# A COMPARATIVE STUDY OF THE EMBRYOLOGY OF *LUDWIGIA* (ONAGRACEAE): CHARACTERISTICS, VARIATION, AND RELATIONSHIPS[1]

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ABSTRACT

Based on our examination of samples representing seven sections, 11 species, and up to 40 characters, we describe the embryological characteristics of the genus *Ludwigia* (Onagraceae). *Ludwigia* represents a phylogenetic line separate from all other Onagraceae, so it occupies a key position in considerations of relationships within the family and between Onagraceae and other families of Myrtales. The typical distinctive 4-nucleate *Oenothera*-type embryo sac was present in all species studied, displaying no distinctive features. The embryo sacs of Myrtales, including Onagraceae and Lythraceae, lack antipodal cells at maturity. This character cannot therefore be used to demonstrate relationships between these families, as suggested by earlier embryologists. Two derived embryological features that are shared by Onagraceae and Lythraceae, however, and might indicate relationships between them are (1) the ubiquitous occurrence of starch grains in the nucellus; and (2) tracheidal exotegmens. Among the embryological features that we examined in *Ludwigia,* seed coat anatomy may be most useful in considering relationships within the genus. For example, the specialized endotestal structure of sect. *Dantia* is found only in members of sect. *Microcarpium,* thus supporting the hypothesis that the two sections are closely related.

Despite the fact that it clearly belongs in the order Myrtales, Onagraceae is very distinctive within that order (Cronquist, 1981; Dahlgren & Thorne, 1984; Johnson & Briggs, 1984). One of the most distinctive aspects of the family is its embryology, and in particular its unique 4-nucleate *Oenothera* type embryo sac (Seshavataram, 1970; Raven, 1979; Tobe & Raven, 1983). Within Onagraceae, the genus *Ludwigia,* consisting of about 80 species found mainly in the tropics and in temperate North America, is the only genus of tribe Jussiaeeae (Raven, 1979). *Ludwigia* has been studied intensively from various points of view, partly because it has been considered to be a primitive group, closest to a prototype of Onagraceae (see Parmentier, 1897; Melchior, 1964; Takhtajan, 1980). Earlier studies of *Ludwigia* have been concerned with biosystematics (Duke, 1955; Schmidt, 1967; Ramamoorthy & Zardini, 1987; Peng, 1982); chromosomal observations (Gregory & Klein, 1960; Kurabayashi et al., 1962; Raven & Tai, 1979); pollen morphology (Skvarla et al., 1975, 1976, 1978); reproductive morphology and anatomy (Eyde, 1977, 1978, 1981); and leaf anatomy (Keating, 1982). These works as a whole have indicated both the intersectional diversity of *Ludwigia* and its distinctiveness as a genus.

Eyde (1981) provided a comprehensive review of the interrelationships of the sections of *Ludwigia*. He suggested, based on his analysis of its characteristics, that *Ludwigia* is the sister group of all other Onagraceae, a concept that is well supported by evidence drawn from many different lines of investigation.

The present study is concerned with the embryological characters of the genus; i.e., the development of anthers, ovules, embryos, seeds, and gametophytes. All of these characters have been poorly investigated in the past. Eleven species representing seven sections, selected to represent the range of variation in the genus, have been examined in detail. This study is intended to illuminate the pattern of variation in embryological characteristics within the genera, and to provide a sound basis for comparisons with other members of the family Onagraceae and the order Myrtales.

## REVIEW OF EARLIER EMBRYOLOGICAL STUDIES

*Anthers and microspores.* Seshavataram (1967, 1970) described these features in *Lud-*

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*wigia octovalvis* (Jacq.) Raven. The anther wall is six-layered and consists of the epidermis, fibrous endothecium, three middle layers, and the tapetum; the tapetum is glandular, and its cells are two-nucleate; cytokinesis in the microspore mother cells is simultaneous. No other details were described.

*Ovule and megagametophyte formation.* Täckholm (1915) reported the presence of the 4-nucleate *Oenothera* type embryo sac in *Ludwigia octovalvis* (Jacq.) Raven (= "*Jussieua* cfr. *villosa* Lam.," "*J.* cf. *suffruticosa* DC."). His report came only a few years after Geerts (1908) first detected this unique type in *Oenothera glazioviana* Mart. (= *O. lamarckiana* de Vries). Subsequent papers dealt with *L. peploides* (HBK.) Raven (= "*Jussieua repens*"; Ishikawa, 1918); *L. adscendens* (L.) Hara (= "*Jussieua repens*"; Maheshwari & Gupta, 1934; Khan, 1942); *L. epilobioides* Maxim. (= "*L. prostrata*"; Ishikawa, 1918); *L. perennis* L. (= "*L. parviflora*"; Maheshwari & Gupta, 1934); and again with *L. octovalvis* (Seshavataram, 1967, 1970). Taken together, these papers indicate that in *Ludwigia* the ovule in anatropous, bitegmic, and crassinucellate; the micropyle is formed by both integuments; the archesporium is one-celled (in most reports) or multi-celled (Khan, 1942); an archesporial cell cuts off the primary parietal cell; the tetrads of megaspores are linear; the micropylar megaspore in the tetrad is functional and develops into a 4-nucleate *Oenothera* type embryo sac; and the chalazal megaspore may also develop to a certain degree, resulting in an additional rudimentary embryo sac in an ovule.

*Fertilization and endosperm.* Porogamous fertilization and Nuclear endosperm formation were reported in *Ludwigia octovalvis* (= "*Jussieua* cfr. *villosa*"; Täckholm, 1915; Seshavataram, 1967, 1970) and *L. adscendens* (= "*Jussieua repens*"; Khan, 1942). Täckholm (1915) stated that the free endosperm nuclei became cellular only at the micropylar region, whereas Seshavataram (1970) observed that the endosperm became absolutely cellular. In this connection, Johansen (1931: 23) implied that *Ludwigia,* which he considered a primitive genus, had a dense and distinctly cellular endosperm as a heritage from its ancestor, whereas *Epilobium* (= "*Zauschneria*"), an advanced genus, had a coenocytic endosperm, or no endosperm at all at maturity.

*Hypostase.* Täckholm (1915) first simply described that a hypostase was differentiated in *Ludwigia octovalvis* (= "*Jussieua* cfr. *villosa*"). A later paper that was concerned with *L. peploides* (= "*Jussieua repens*") and *L. epilobioides* (= "*L. prostrata*"; Ishikawa, 1918) reported that a hypostase was absent. Later, Johansen (1928a, 1928b) declared that there was no hypostase in *Ludwigia,* based on the observations of a *Ludwigia* that he called *L. mullertii.* He contrasted its absence in *Ludwigia* with its presence in such genera as *Oenothera, Gaura,* and *Clarkia.* Johansen explained the function of the hypostase (and the epistase) as follows: "They serve to stabilize the water balance of the resting seed over the long period of dormancy during the hot dry season." Maheshwari and Gupta (1934) did not observe the hypostase in *L. perennis* L. (= "*L. parviflora*") and *L. adscendens* (L.) Hara (= "*Jussieua repens*") either. In contrast, Khan (1942) reported that in *L. adscendens* (= "*Jussieua repens*") neither a hypostase nor an epistase is present in the young ovules, but that a conspicuous hypostase was formed subsequently. Seshavataram (1967) confirmed the presence of a hypostase in the older stages of development of the ovule of *L. octovalvis.*

*Starch grains in the nucellus.* Ishikawa (1918) first described starch grains in the nucellus of Onagraceae. He reported that the starch grains were rather infrequent in *L. epilobioides* (= "*L. prostrata*") and few in *L. adscendens,* in contrast with their abundance in *Oenothera, Gaura,* and *Circaea.* Because of his belief that *Ludwigia* lacked a hypostase, Ishikawa inferred that its presence was correlated with a lack of starch grains in the nucellus.

*Embryogeny.* Only the Onagrad type of embryogeny has been recorded in *Ludwigia.* Reports include those of Souèges (1935) for *L. palustris* (L.) Elliott, Khan (1942) for *L. adscendens*; and Seshavataram (1967, 1970) for *L. octovalvis.*

*Seed coat.* Corner (1976) first gave general descriptions of the seed coat histology of *Ludwigia* (= "*Jussieua*"), based on his observation of *L. peruviana* (L.) Hara (= "*Jussieua peruviana*"). The testa is two-layered with the endotesta composed of crystal cells that are lignified and stellately lobed with thickened inner and radial walls. The tegmen is also two-layered with the fibrous exotegmen. Eyde (1978) provided general descriptions of the seed coat histology of species of seven sections of *Ludwigia,* including *L. peruviana.*

This review of the available literature indicates clearly that, although quite a few reports on the embryology of *Ludwigia* have been published,

TABLE 1. Vouchers of the *Ludwigia* species used.

| Species | Vouchers |
|---|---|
| Sect. *Myrtocarpus* | |
| *L. peruviana* (L.) Hara | Australia. Mascot District, Sydney, *B. G. Briggs 7143, 7226* (NSW). |
| Sect. *Macrocarpon* | |
| *L. lagunae* (Morong) Hara | Paraguay. Asunción, Luque, *T. P. Ramamoorthy 1019* (MO). Paraguay. Caaguazú, *T. P. Ramamoorthy 1097* (MO). |
| *L. bonariensis* (Michx.) Hara | Argentina. Buenos Aires, *T. P. Ramamoorthy 1005* (MO). Argentina. Tucumán, *T. P. Ramamoorthy 1015* (MO). |
| Sect. *Ludwigia* | |
| *L. maritima* Harper | USA. South Carolina, Colleton Co., *C. Peng 3920* (MO). |
| *L. virgata* Michx. | USA. Alabama, Mobile Co., *H. Tobe s.n.* (MO). |
| Sect. *Microcarpium* | |
| *L. lanceolata* Elliott | USA. Georgia, McIntosh Co., *C. Peng 4139* (MO). |
| *L. glandulosa* Walter subsp. *glandulosa* | USA. Louisiana, Cameron Parish, *C. Peng 4367* (MO). |
| Sect. *Dantia* | |
| *L. repens* Forster | USA. Florida, Lee Co., *C. Peng 4290* (MO). |
| *L. arcuata* Walter | USA. Florida, Hillsborough Co., *C. Peng 4320* (MO). |
| Sect. *Seminuda* | |
| *L. leptocarpa* (Nutt.) Hara | Brazil. Bentos, Santa Catarina, *T. P. Ramamoorthy 1134* (MO). USA. Georgia, Barnwell Co., *R. W. Dolan 1* (MO). |
| Sect. *Oligospermum* | |
| *L. peploides* (Kunth) Raven | USA. Missouri, St. Louis Co., *H. Tobe s.n.* (MO). |

there still remain several gaps in the available information. Also, the discrepancies in literature regarding the ovular archesporium, the endosperm, and the hypostase remain to be resolved.

## MATERIALS AND METHODS

The 11 species examined in this study are listed in Table 1, together with voucher informa-

TABLE 2. A summary of embryological data on anthers and microspores.

| Taxa | Thickness of Anther Wall | Type of Anther Wall Development | Epidermis | Endothecium | Tapetum | Nuclear Number of Tapetal Cell | Delimitation of Microspores | Microspore Tetrads[1] | Cell Number in Mature Pollen |
|---|---|---|---|---|---|---|---|---|---|
| Sect. *Myrtocarpus* | | | | | | | | | |
| *L. peruviana* | 5–6 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | usually tetrahedral/ rarely decussate/ very rarely isobilateral | 2-celled |

TABLE 2. Continued.

| Taxa | Thickness of Anther Wall | Type of Anther Wall Development | Epidermis | Endothecium | Tapetum | Nuclear Number of Tapetal Cell | Delimitation of Microspores | Microspore Tetrads[1] | Cell Number in Mature Pollen |
|---|---|---|---|---|---|---|---|---|---|
| Sect. *Macrocarpon* | | | | | | | | | |
| *L. lagunae* | 5 layers | Basic | persistent | fibrous | glandular | 2-nucleate | — | usually tetrahedral/very occasional decussate/rarely isobilateral | 2-celled |
| *L. bonariensis* | 5–6 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | — | 2-celled |
| Sect. *Ludwigia* | | | | | | | | | |
| *L. maritima* | — | — | — | — | — | — | — | — | 2-celled |
| *L. virgata* | 5–6 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | — | 2-celled |
| Sect. *Microcarpium* | | | | | | | | | |
| *L. lanceolata* | 5 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | usually tetrahedral/occasionally decussate | 2-celled |
| *L. glandulosa* subsp. *glandulosa* | 5 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | usually tetrahedral/very occasionally decussate | 2-celled |
| Sect. *Dantia* | | | | | | | | | |
| *L. repens* | 5 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | usually tetrahedral/occasionally decussate | 2-celled |
| *L. arcuata* | 5–6 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | — | 2-celled |
| Sect. *Seminuda* | | | | | | | | | |
| *L. leptocarpa* | 5 layers | Basic | persistent | fibrous | glandular | 2-nucleate | simultaneous | nearly always tetrahedral/rarely decussate | 2-celled |
| Sect. *Oligospermum* | | | | | | | | | |
| *L. peploides* | 5–6 layers | Basic | persistent | fibrous | glandular | 2-nucleate | — | — | 2-celled |

[1] Based on 50 selected tetrads. Expressions for frequency follow Schmid (1982).

tion. The samples of flower buds and fruits in various stages of development were fixed with FAA (5 parts stock formalin; 5 parts glacial acetic acid; 90 parts 70% ethanol). Subsequently, they were dehydrated through a *t*-butyl alcohol series, and then embedded in Paraplast with melting point 57–58°C for microtome sectioning. Serial sections cut 5–7 μm in thickness were stained with Heidenhain's hematoxylin, safranin, and fast green FCF, and mounted in Histoclad.

Observations of microtome sections were made with a Zeiss Standard microscope equipped with a phase-contrast condenser. The photomicrographs were mostly taken using an Iris diaphragm for bright-field observations. In some, annular stops were used for the phase-contrast observations in order to show the location of starch grains in the nucellus (e.g., Figs. 8–12, 15, 16).

## RESULTS

Embryological data concerning all species studied are summarized and compared in Tables 2–6. A discussion of these characteristics is presented in the following pages.

### ANTHERS AND MICROSPORES (TABLE 2)

The thickness of anther wall varies from five to six cell layers (Fig. 1). There is no conspicuous difference in thickness from species to species, nor from section to section. The anther wall is composed of an epidermis, an endothecium, two or three middle layers, and a tapetum (Fig. 1). Following the definitions of types by Davis (1966: 8–11), the wall formation of all the species studied conforms to the Basic type. In it, the middle layers have a common histogenetic origin with both the endothecium and the tapetum (Fig. 1).

As the anther develops, the middle layers are completely crushed (Fig. 2). The epidermis is persistent and remains uncrushed until the time of anther dehiscence; in some species such as *Ludwigia leptocarpa* (Fig. 3) and *L. peploides,* the epidermal cells are more or less flattened, but in most other species, they are enlarged as much as the endothecial cells (Fig. 4). The endothecium always develops fibrous thickenings, as shown by Eyde (1977). The tapetum is glandular and its cells become two-nucleate.

Meiosis in the microspore mother cells is accompanied by simultaneous cytokinesis (see arrow in Fig. 2), as recorded in *L. octovalvis* (Seshavataram, 1967, 1970). The arrangement of microspores in a tetrad is usually tetrahedral, occasionally decussate, and rarely isobilateral throughout the genus; there is no conspicuous difference in the frequencies of the different types of arrangement from species to species. The pollen grains are two-celled when shed (Fig. 5).

### OVULES (TABLE 3)

The characteristics of the ovule are remarkably consistent throughout the genus: the ovule is anatropous, crassinucellate, and bitegmic. The inner integument is always two-layered. The outer integument is two-layered in most species but is three-layered in its basal portion in *Ludwigia peploides* (sect. *Oligospermum*). The distinctive mode of initiation of the integuments in *L. peploides* is discussed in a separate paper (Tobe & Raven, 1985). The micropyle is always formed by both integuments.

### MEGAGAMETOPHYTE FORMATION AND NUCELLUS (TABLE 4)

The archesporium is uniformly one-celled (Fig. 6), contrary to a report by Khan (1942), who wrote that it looked multicellular in *Ludwigia adscendens.* Very rarely, more than one megaspore mother cell (archesporial cell) is found in

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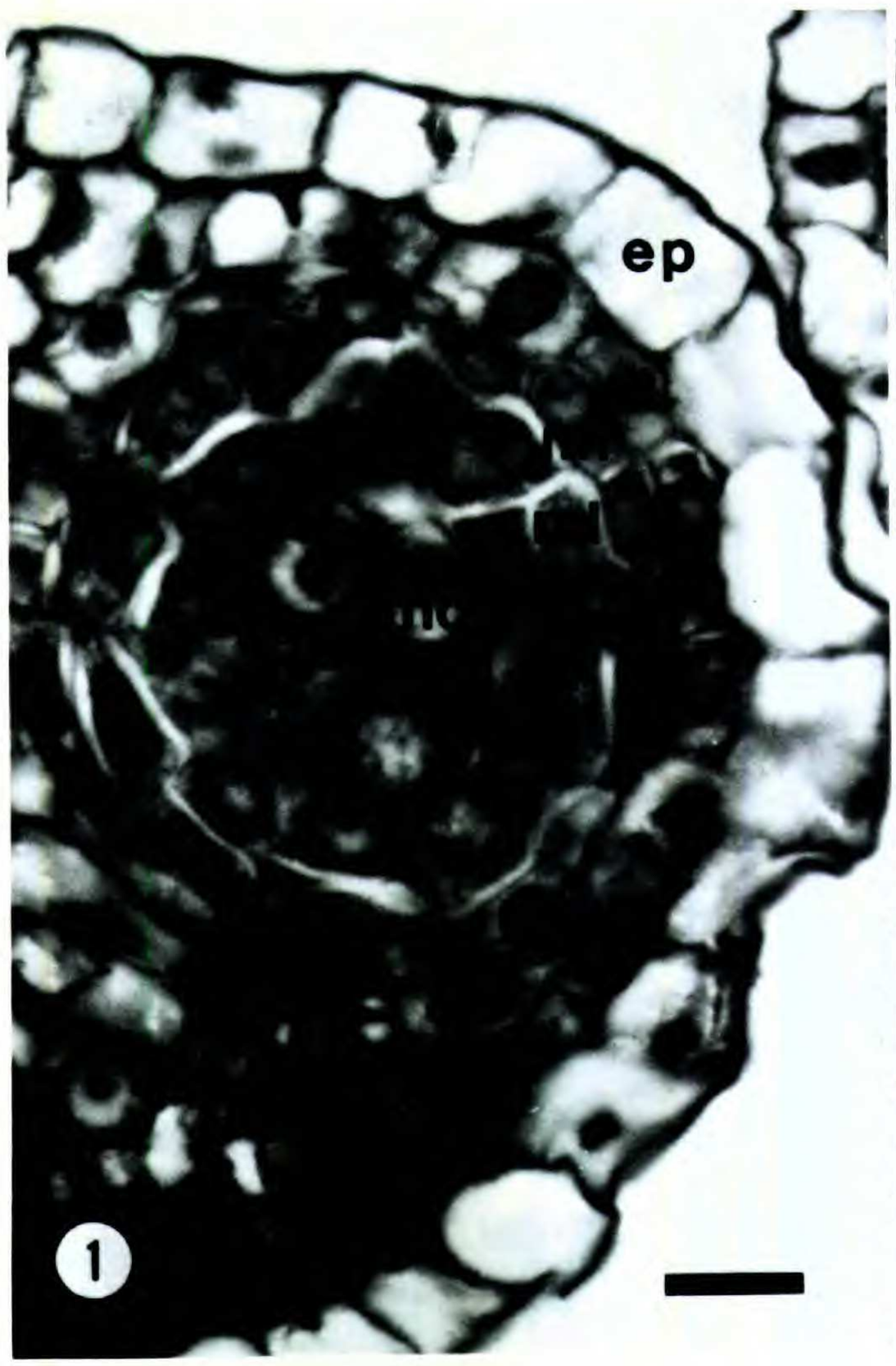
FIGURES 1–5. Anthers and microspores of *Ludwigia.*—1. *L. leptocarpa.* Transverse section (TS) of a young anther. The wall is 5-layered, consisting of epidermis (ep), endothecium (et), two middle layers (ml), and tapetum (t) (mc = microspore mother cell). Scale = 10 μm.—2. *L. bonariensis.* TS of an older anther showing collapsed middle layers and 2-nucleate glandular tapetal cells (t); epidermis (ep), endothecium (et). An arrow indicates cell at telophase of meiosis II. Scale = 50 μm.—3. *L. leptocarpa.* TS of a dehisced anther. The wall is composed of a flattened persistent epidermis (ep) and a fibrous endothecium (et). Scale = 100 μm.—4. *L. linearis* (*Dille 420,* MO). TS of a dehisced anther. The wall is composed of well-developed persistent epidermis (ep) and a fibrous endothecium (et). Scale = 100 μm.—5. *L. leptocarpa.* Two-celled pollen grain artificially separated from the normal tetrad formation found at dehiscence; stained with 1% aceto-carmine. Arrows indicate a large vegetative nucleus and a smaller generative one. Scale = 50 μm.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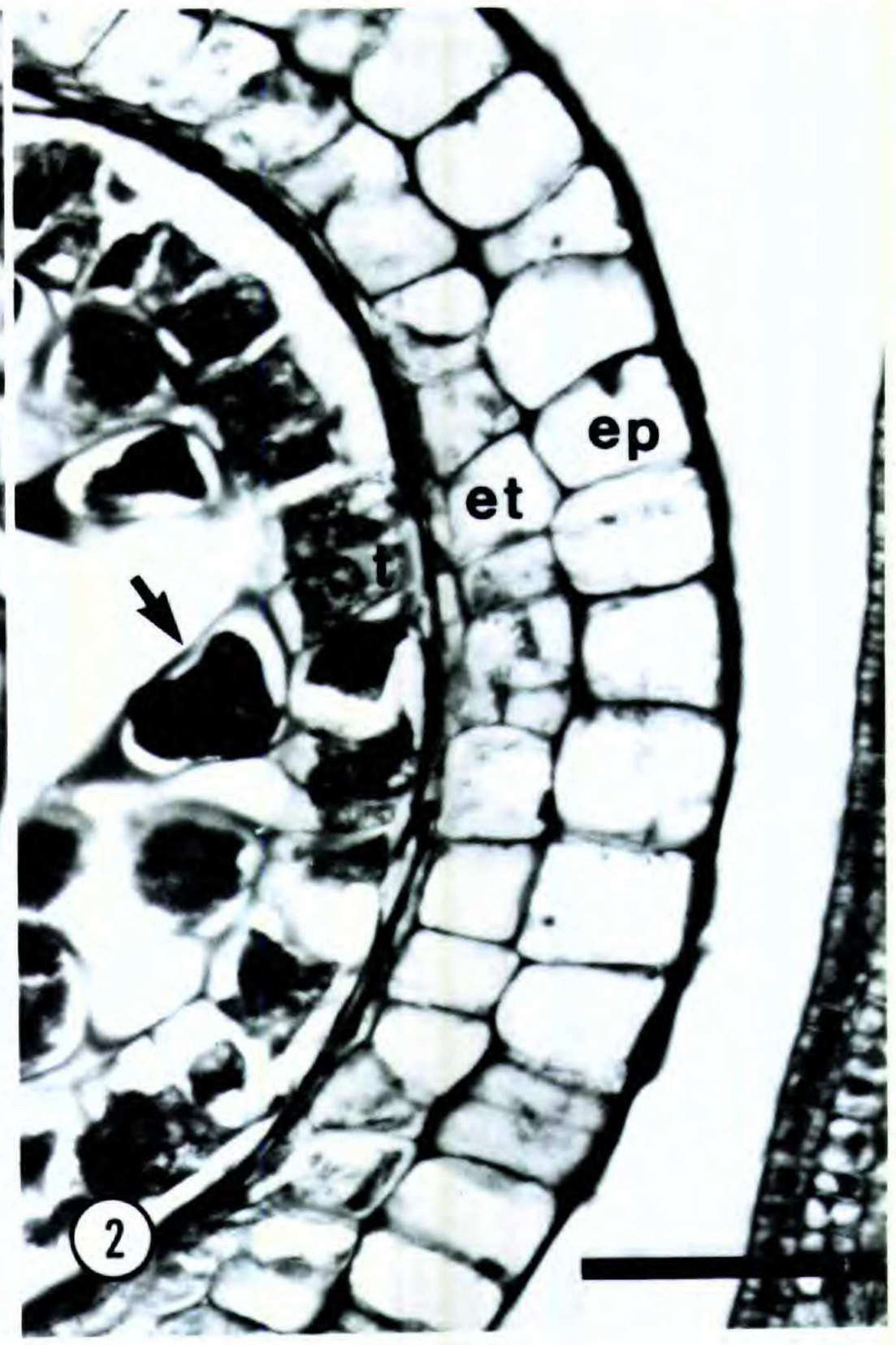
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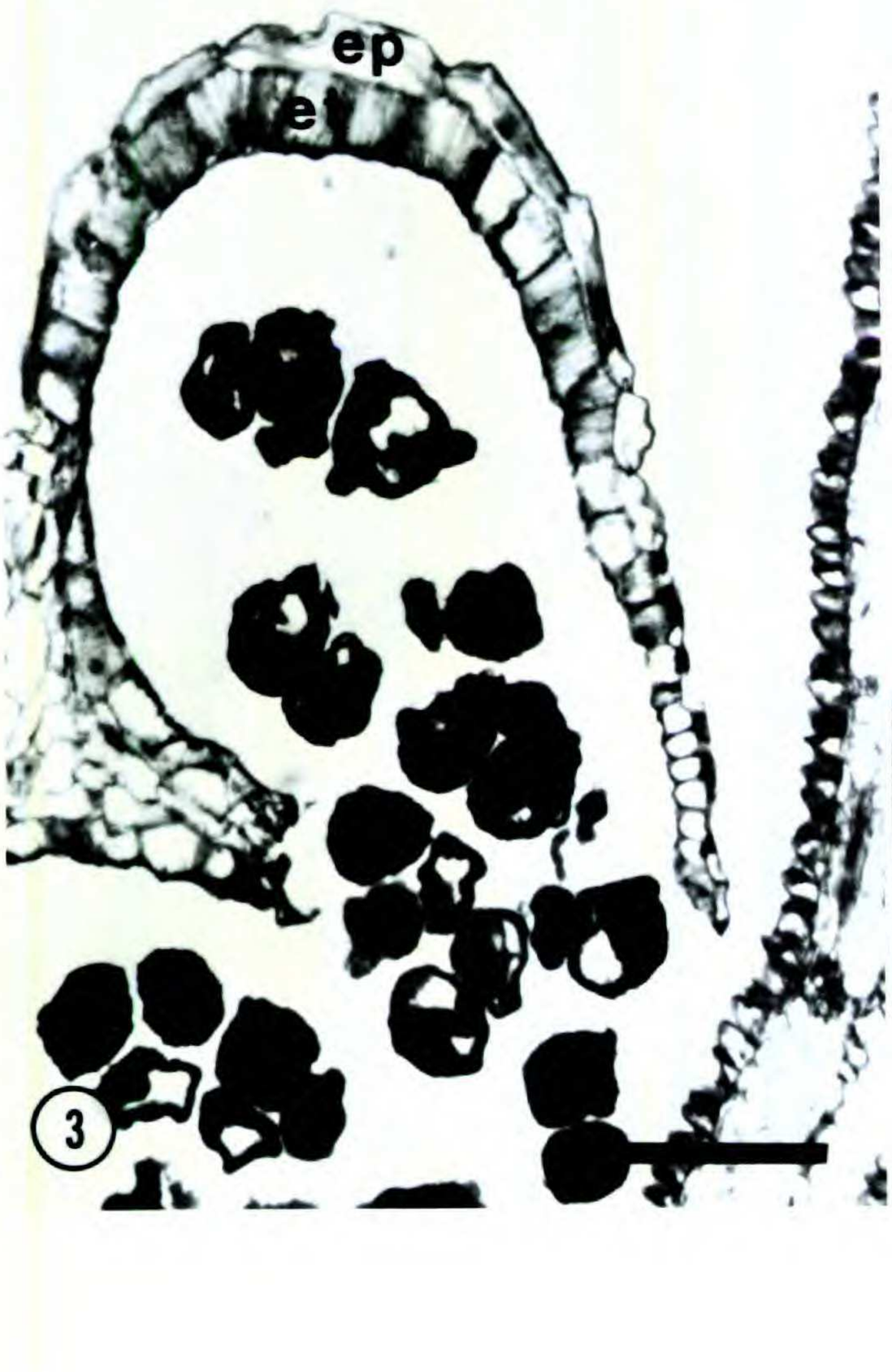
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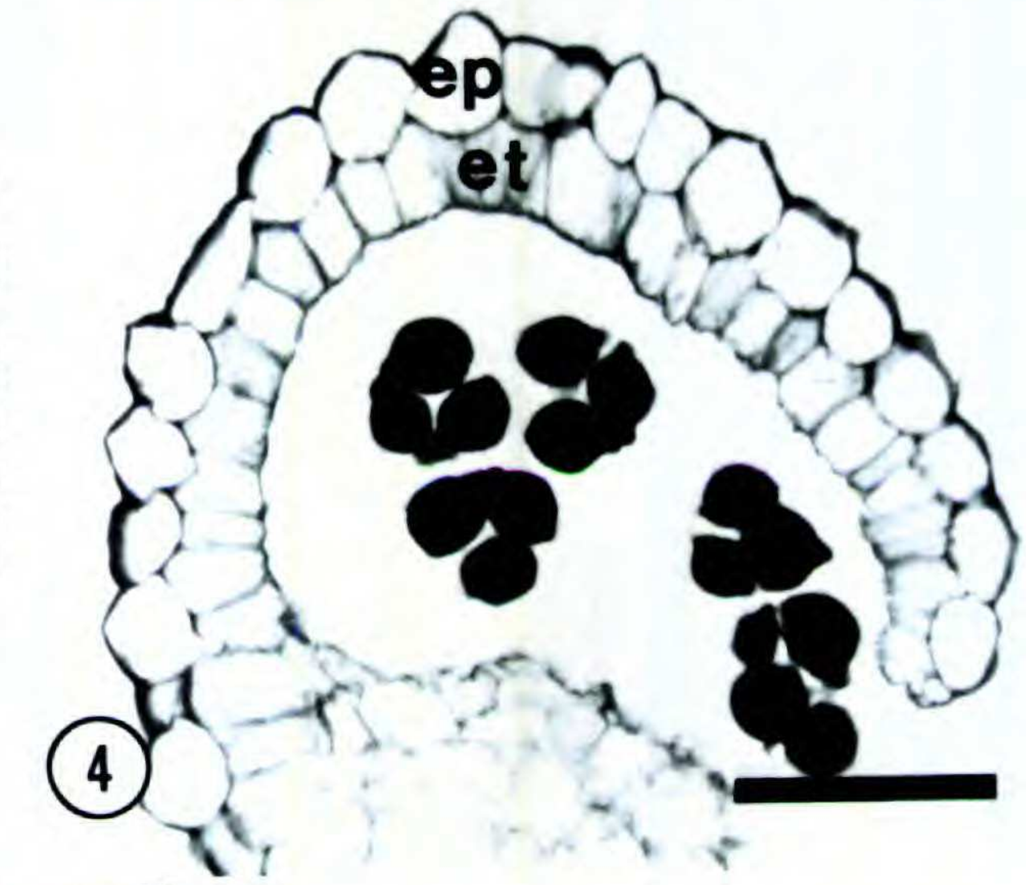
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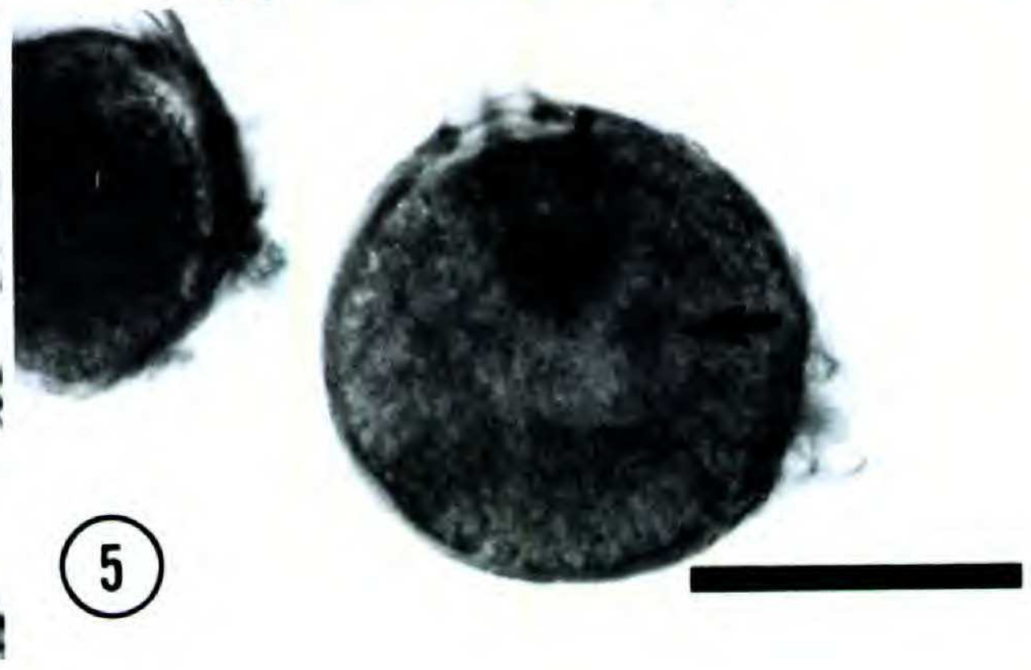
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TABLE 3. A summary of embryological data on ovules. Abbreviations: ii, inner integument; oi, outer integument.

| Taxa | Ovule Type | Nature of Nucellus | Number of Integu-ments | Thickness of ii | Thickness of oi | Formation of Micropyle | Starch Grains in the Nucellus |
|---|---|---|---|---|---|---|---|
| Sect. *Myrtocarpus* | | | | | | | |
| *L. peruviana* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| Sect. *Macrocarpon* | | | | | | | |
| *L. lagunae* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| *L. bonariensis* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| Sect. *Ludwigia* | | | | | | | |
| *L. maritima* | – | – | 2 | – | – | – | – |
| *L. virgata* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| Sect. *Microcarpium* | | | | | | | |
| *L. lanceolata* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| *L. glandulosa* subsp. *glandulosa* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| Sect. *Dantia* | | | | | | | |
| *L. repens* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| *L. arcuata* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| Sect. *Seminuda* | | | | | | | |
| *L. leptocarpa* | anatropous | crassinucellate | 2 | 2-layered | 2-layered | ii and oi | present |
| Sect. *Oligospermum* | | | | | | | |
| *L. peploides* | anatropous | crassinucellate | 2 | 2-layered | 2(–3)-layered | ii and oi | present |

TABLE 4. A summary of embryological data on megagametophyte formation.

| Taxa | Number of Archesporial Cells | Thickness of Parietal Tissue | Arrangement of Megaspore Tetrad | Position of Functional Megaspore | Type of Embryo Sac Formation | Number of Nuclei in Mature Embryo Sac | Shape of Mature Embryo Sac |
|---|---|---|---|---|---|---|---|
| Sect. *Myrtocarpus* | | | | | | | |
| *L. peruviana* | 1 | 4–5 layered | linear | micropylar | *Oenothera* | 4 | ellipsoidal |
| Sect. *Macrocarpon* | | | | | | | |
| *L. lagunae* | 1 | 4–6 layered | linear | micropylar | *Oenothera* | 4 | ovoid |
| *L. bonariensis* | 1 | 4–5 layered | linear | micropylar | *Oenothera* | 4 | ovoid |
| Sect. *Ludwigia* | | | | | | | |
| *L. maritima* | — | — | — | — | — | — | — |
| *L. virgata* | 1 | 4–5 layered | linear | micropylar | *Oenothera* | 4 | ovoid |
| Sect. *Microcarpium* | | | | | | | |
| *L. lanceolata* | 1 | 4–5 layered | linear | micropylar | *Oenothera* | 4 | ellipsoidal |
| *L. glandulosa* subsp. *glandulosa* | 1 | 4–6 layered | linear | micropylar | *Oenothera* | 4 | ellipsoidal |
| Sect. *Dantia* | | | | | | | |
| *L. repens* | 1 | 4–5 layered | linear | micropylar | *Oenothera* | 4 | ellipsoidal |
| *L. arcuata* | 1 | 4–5 layered | linear | micropylar | *Oenothera* | 4 | ellipsoidal |
| Sect. *Seminuda* | | | | | | | |
| *L. leptocarpa* | 1 | 3–4 layered | linear | micropylar | *Oenothera* | 4 | spherical |
| Sect. *Oligospermum* | | | | | | | |
| *L. peploides* | 1 | 5–6 layered | linear | micropylar | *Oenothera* | 4 | ellipsoidal |

a given nucellus. This condition was not characteristic of any species examined. Each archesporial cell divides periclinally into two: the upper primary parietal cell and the lower primary sporogenous cell. The primary parietal cell further divides periclinally (Fig. 7). Its daughter cells repeatedly divide periclinally and anticlinally, which results in a three- to six-layered parietal tissue being formed above the young embryo sac. The primary sporogenous cell directly develops into a megaspore mother cell (Figs. 7, 8) that later undergoes meiosis, forming megaspores (Figs. 9–11).

Heterotypic division in the meiosis of the megaspore mother cell results in the production of a dyad usually composed of a larger micropylar and a smaller chalazal cell (Figs. 9, 10). This contrasts sharply with the situation in most angiosperms, in which a dyad consisting of a smaller micropylar cell and a larger chalazal cell is common. The subsequent homotypic division usually occurs in both micropylar and chalazal cells, resulting in the formation of a linear tetrad of megaspores. Occasionally, it occurs only in the micropylar cell, in which case a linear triad of megaspores is formed. In either a tetrad or a triad, the micropylar megaspore is always the largest cell, and the functional one (Fig. 11). This megaspore enlarges, while the others degenerate (Fig. 12). Occasionally, we have observed an enlarging chalazal megaspore opposite the enlarged micropylar megaspore, a condition that was reported repeatedly in earlier works (e.g., Ishikawa, 1918). We have never observed twin mature embryo sacs derived from both the micropylar and the chalazal megaspore, however, nor have we ever seen a single mature embryo sac derived from the chalazal megaspore alone.

The functional megaspore undergoes two successive nuclear divisions, forming a megagametophyte. As has often been indicated in the earlier works, the nucleus of the functional megaspore moves toward the micropylar side before the first nuclear division; the two nuclei resulting from its first meiotic division remain on the micropylar side (see Fig. 13), with one slightly above the other. In the second nuclear division, the upper nucleus divides horizontally, whereas the lower one divides vertically (Fig. 13). It appears that the two nuclei derived from the upper nucleus develop into the two synergids, whereas those derived from the lower nucleus develop into the egg and the polar nucleus, as mentioned by Khan (1942). Thus, megagametophyte formation in *Ludwigia* conforms exactly to the *Oenothera*-type: the organized embryo sac contains the egg, two synergids and one polar nucleus, and lacks any antipodal cells (Figs. 15, 16). Very rarely an embryo sac with more than four nuclei is formed when other megaspores, which normally would have degenerated, are released from the nucellus into the embryo sac (Fig. 14). Such an aberrant embryo sac clearly does not indicate any similarity to any other type.

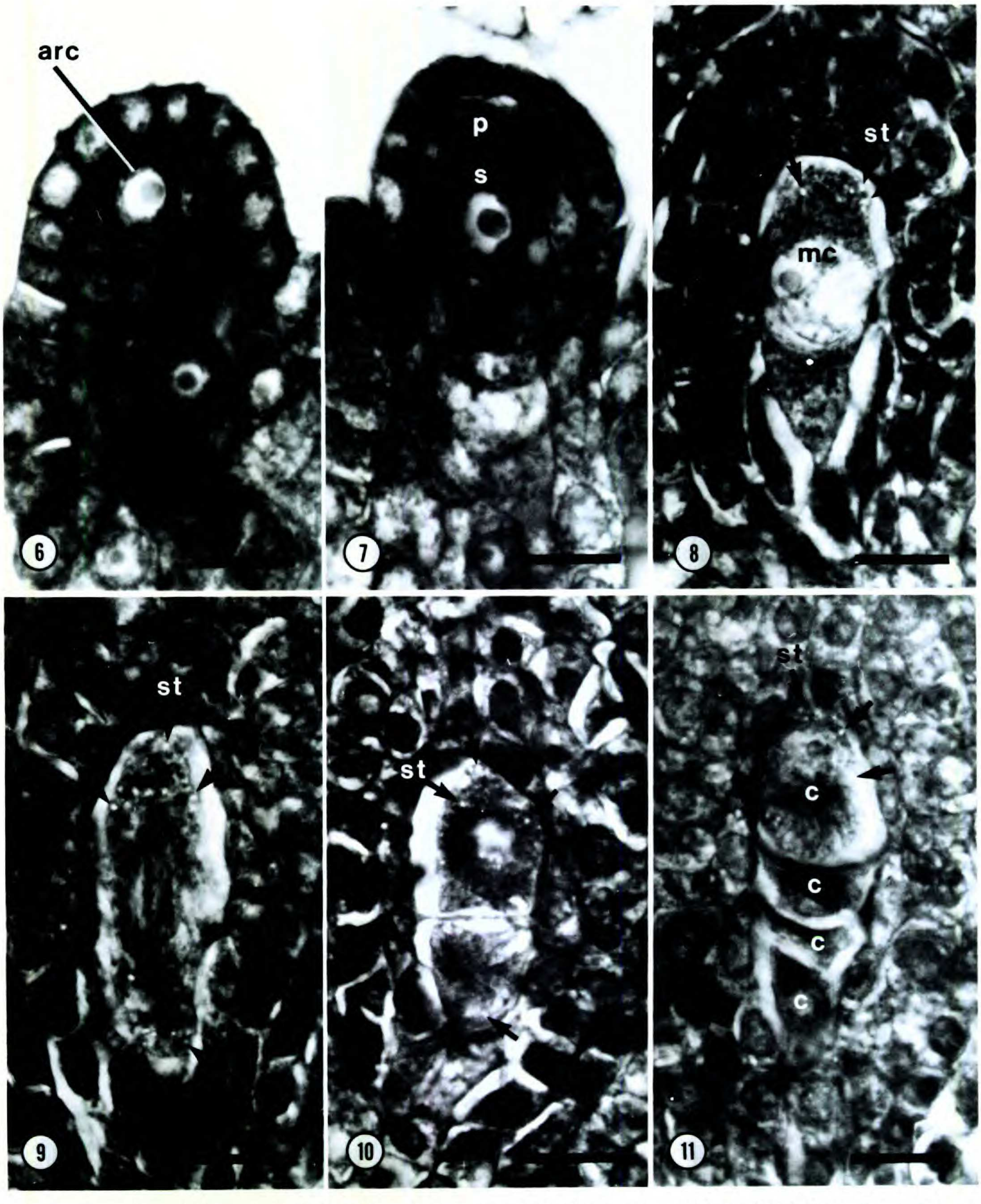
In *Ludwigia,* the mature embryo sacs are diverse in shape, varying from spherical or ovoid to ellipsoidal. There seem to be no significant differences in the shape of the mature embryo sac from one species to another, however.

During megasporogenesis and megagametogenesis, no particular specialization occurs in the nucellar tissue. Apical epidermal cells of a nucellus do not divide periclinally. This character is significant because the more or less related members of the Myrtales (e.g., Combretaceae) show remarkable periclinal divisions of the nucellar apical epidermal cells to form a nucellar cap (see Venkateswarlu & Rao, 1972).

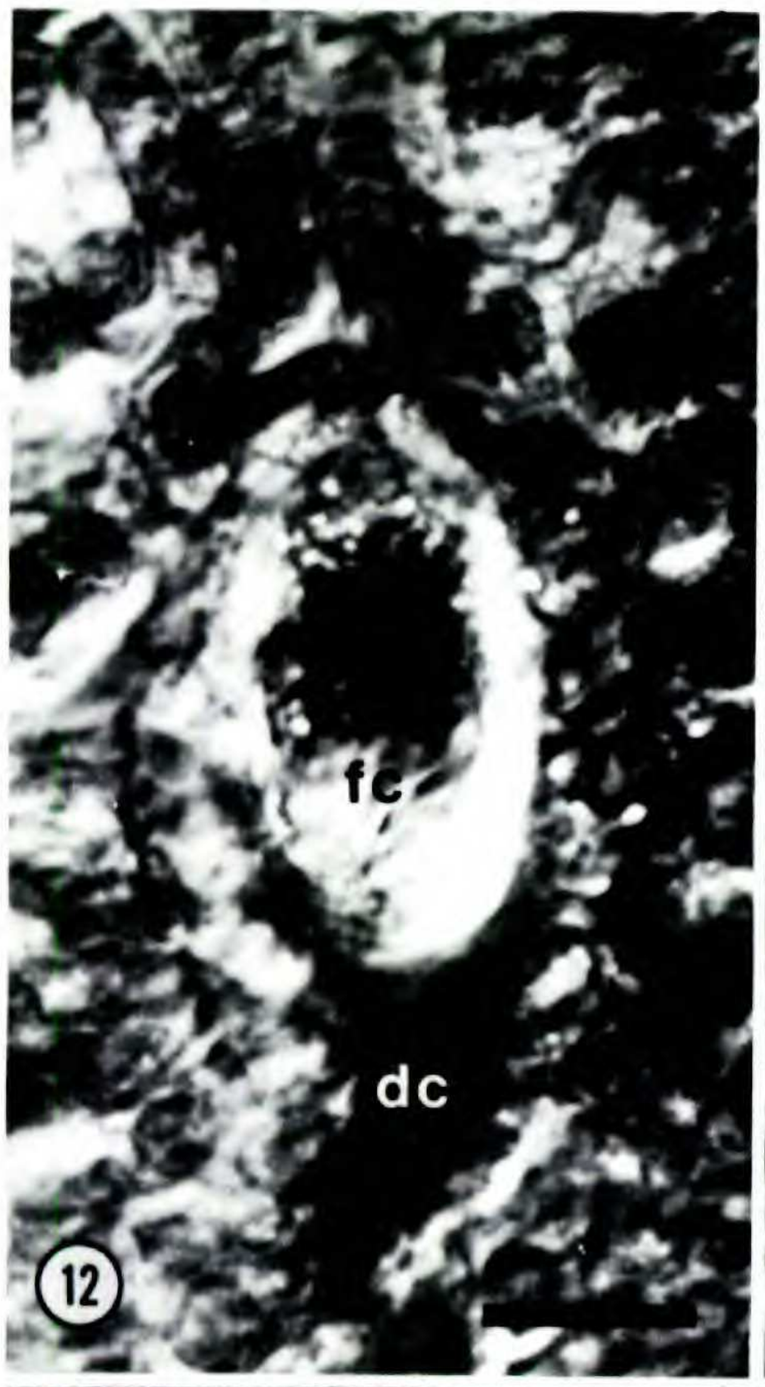
## STARCH GRAINS (TABLE 3)

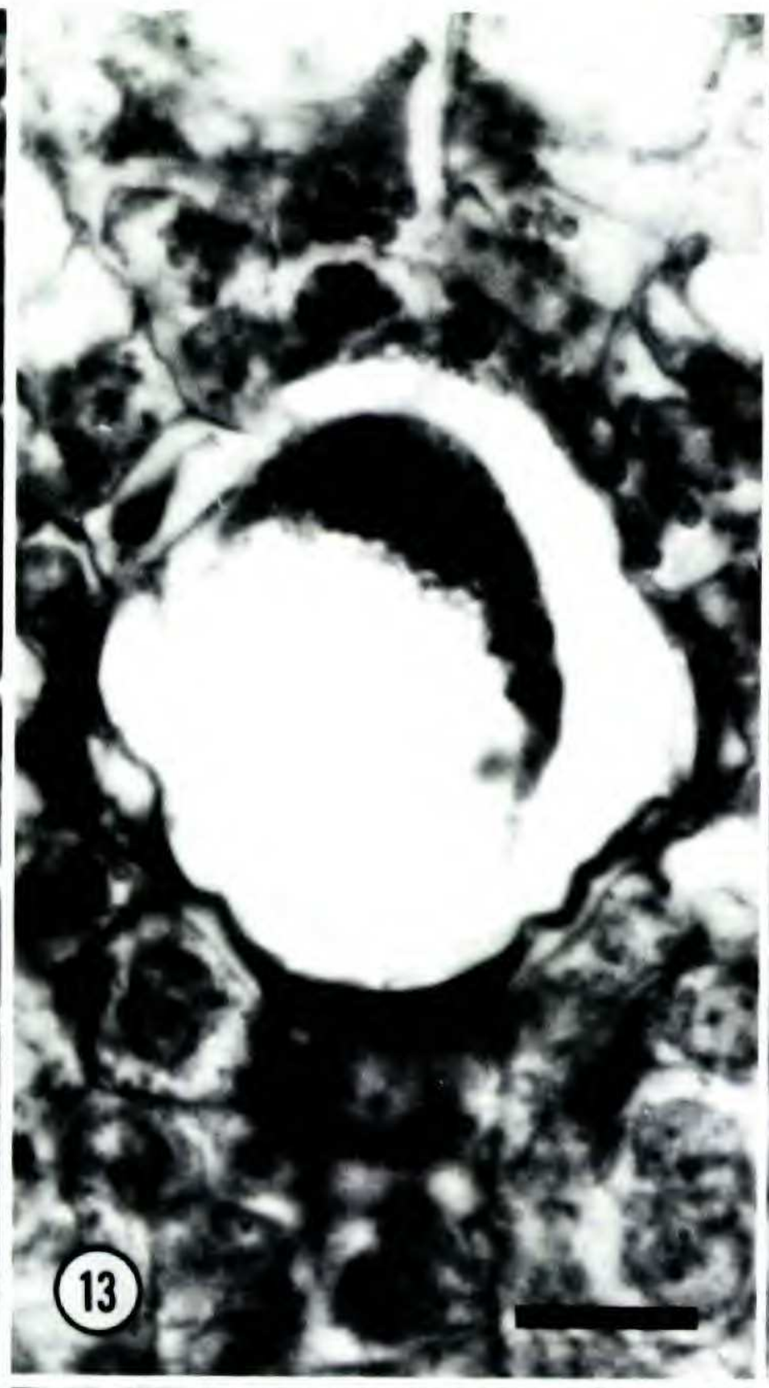
The accumulation of starch grains in the nucellar tissues of all species of *Ludwigia* is conspicuous, contrary to the observation of Ishikawa (1918). In some species—e.g., *L. virgata*—it is less conspicuous than in others. In the details of this process of starch accumulation, *Ludwigia* is exactly similar to *Epilobium* (Rodkiewicz & Bednara, 1974). Starch grains first appear on the upper, or micropylar side, of the megaspore mother cell (Fig. 8). During meiosis I, they are observed both on the micropylar and on the chalazal side, more abundantly on the former (Fig. 9). In the dyad stage, the starch grains of the upper cell are localized on the micropylar side, whereas those of the lower cell are restricted to the chalazal side (Fig. 10). In the tetrad, the functional megaspore, which is micropylar, has starch most abundantly on the micropylar side, while they disappear for the most part from the chalazal megaspore (Fig. 11). The two intervening megaspores have no starch grains.

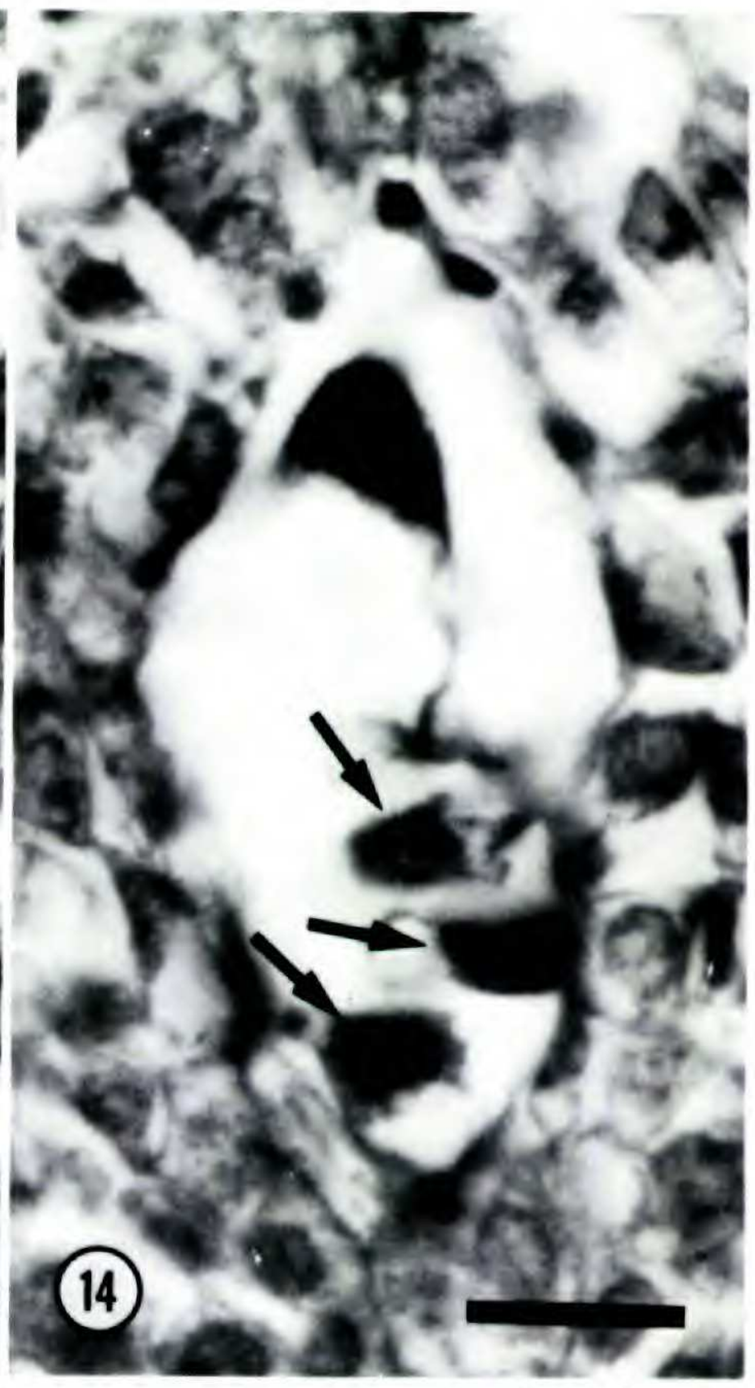
At the functional megaspore stage numerous starch grains are observed not only within the functional megaspore but also in the nucellar tissue surrounding it (Fig. 12). As the ovule grows, more and more starch grains are accumulated in

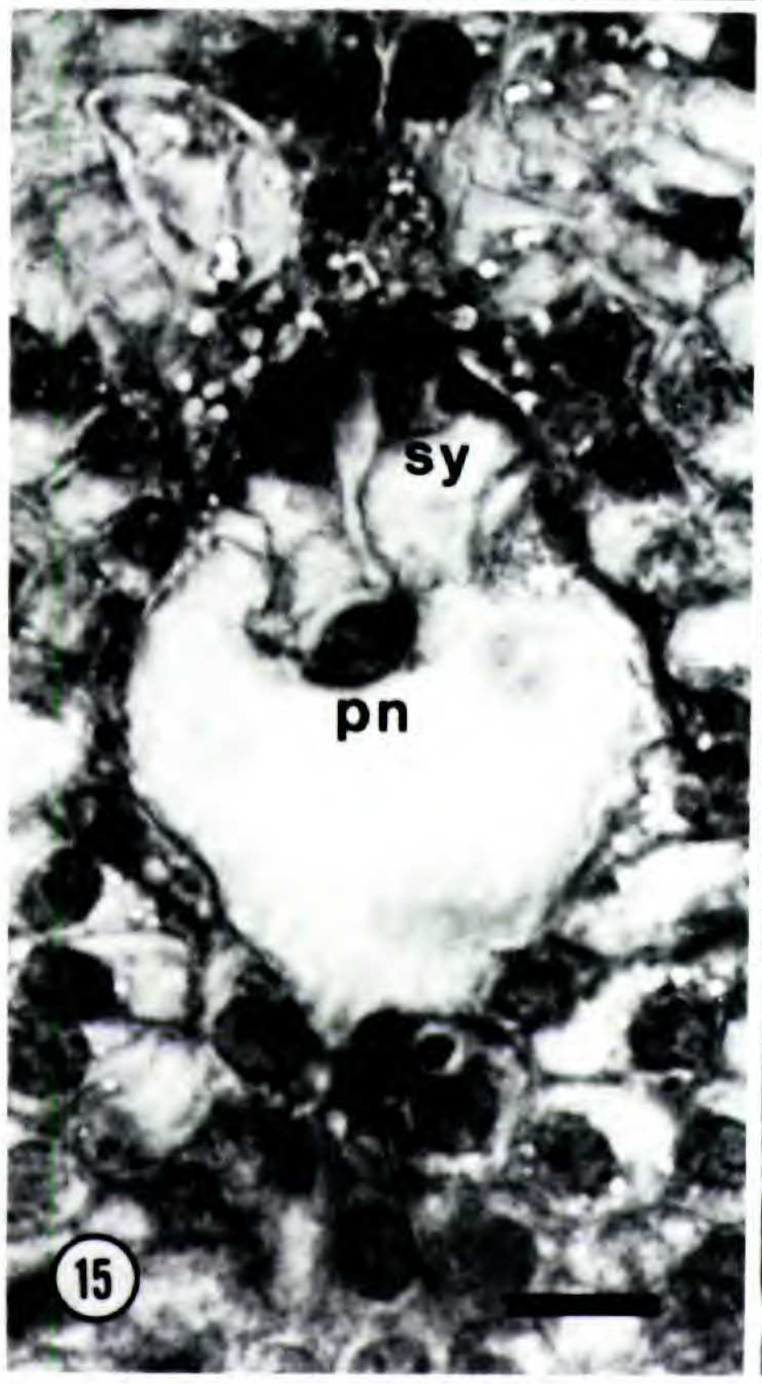


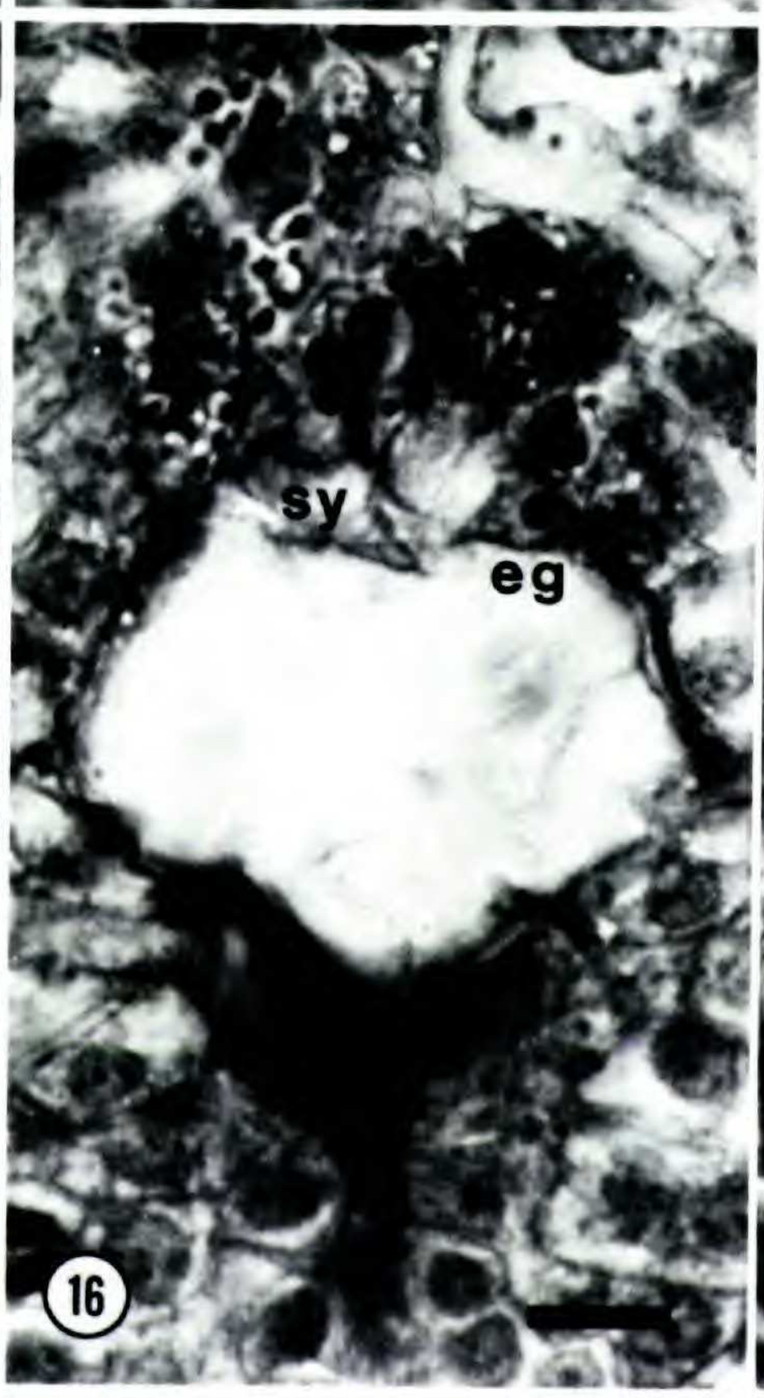

FIGURES 6–11. Archesporium and megasporogenesis in *Ludwigia arcuata*. Longitudinal sections of young ovules from the archesporial cell stage to the megaspore tetrad stage. Arrows in 8–11 point out the location of plastids synthesizing starch grains (st). Scales = 10 μm.–6. Archesporial cell stage. Note that the archesporium (arc) is one-celled.—7. Primary sporogenous cell stage; primary parietal cell (p), primary sporogenous cell (s).—8. Megaspore mother cell stage; megaspore mother cell (mc).—9. Telophase of meiosis I.—10. Megaspore dyad stage.—11. Megaspore tetrad stage. Note that the micropylar megaspore (c) is larger than the lower three (c), and that starch grains are localized only in the micropylar megaspore.





FIGURES 12–17. Megagametogenesis and fertilization in *Ludwigia*. Longitudinal sections of older and fertilized ovules.—12. *L. arcuata*. Functional megaspore stage; functional megaspore (fc); remnant of degenerate megaspores (dc). Starch grains are scattered in the functional megaspore and in the nucellar tissue around it. Scale = 10 μm.—13. *L. peruviana*. Two-nucleate embryo sac stage. Note that the upper of the two nuclei is dividing horizontally and the lower vertically. Scale = 10 μm.—14. *L. virgata*. Unusual embryo sac composed of five nuclei, the lower (cellular) nuclei (arrow) being megaspores that should have degenerated. Scale = 10 μm.—15 and 16. *L. peruviana*. Two successive sections of a mature embryo sac consisting of an egg (eg), two synergids (sy), and one polar nucleus (pn). Note that numerous starch grains are scattered in the nucellar tissue. Scales = 10 μm.—17. *L. repens*. Fertilized ovule stage showing a remnant of a pollen tube (pol) penetrating into the micropyle. Scale = 20 μm.

TABLE 5. A summary of embryological data on fertilization, endosperm, embryo, and seed.

| Taxa | Path of Pollen Tube | Type of Endosperm Formation | Cellular Endosperm | Endosperm in Mature Seed | Type of Embryogeny | Hypostase |
|---|---|---|---|---|---|---|
| Sect. *Myrtocarpus* | | | | | | |
| *L. peruviana* | porogamous | Nuclear | formed | absent | — | present |
| Sect. *Macrocarpon* | | | | | | |
| *L. lagunae* | porogamous | Nuclear | formed | absent | — | present |
| *L. bonariensis* | porogamous | Nuclear | formed | absent | Onagrad | present |
| Sect. *Ludwigia* | | | | | | |
| *L. maritima* | porogamous | Nuclear | formed | absent | Onagrad | present |
| *L. virgata* | porogamous | Nuclear | — | — | — | — |
| Sect. *Microcarpium* | | | | | | |
| *L. lanceolata* | porogamous | Nuclear | formed | absent | — | present |
| *L. glandulosa* subsp. *glandulosa* | porogamous | Nuclear | formed | absent | Onagrad | present |
| Sect. *Dantia* | | | | | | |
| *L. repens* | porogamous | Nuclear | formed | absent | — | present |
| *L. arcuata* | porogamous | Nuclear | formed | absent | — | present |
| Sect. *Seminuda* | | | | | | |
| *L. leptocarpa* | porogamous | Nuclear | formed | absent | — | present |
| Sect. *Oligospermum* | | | | | | |
| *L. peploides* | porogamous | Nuclear | formed | absent | Solanad | present |

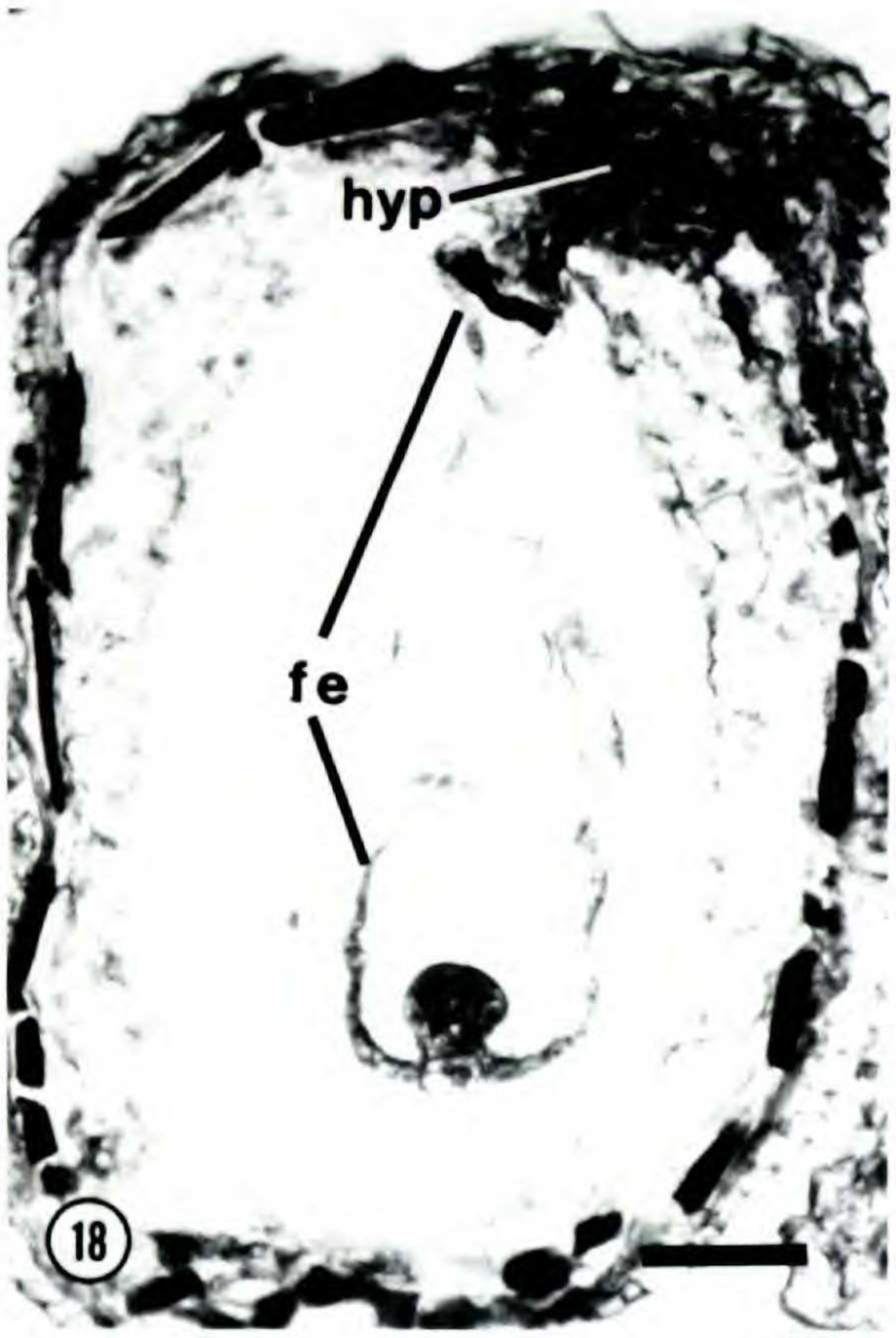
both the embryo sac and the nucellar tissue (Figs. 15, 16). Ultimately, starch grains are found abundantly even within the cells of the proembryo.

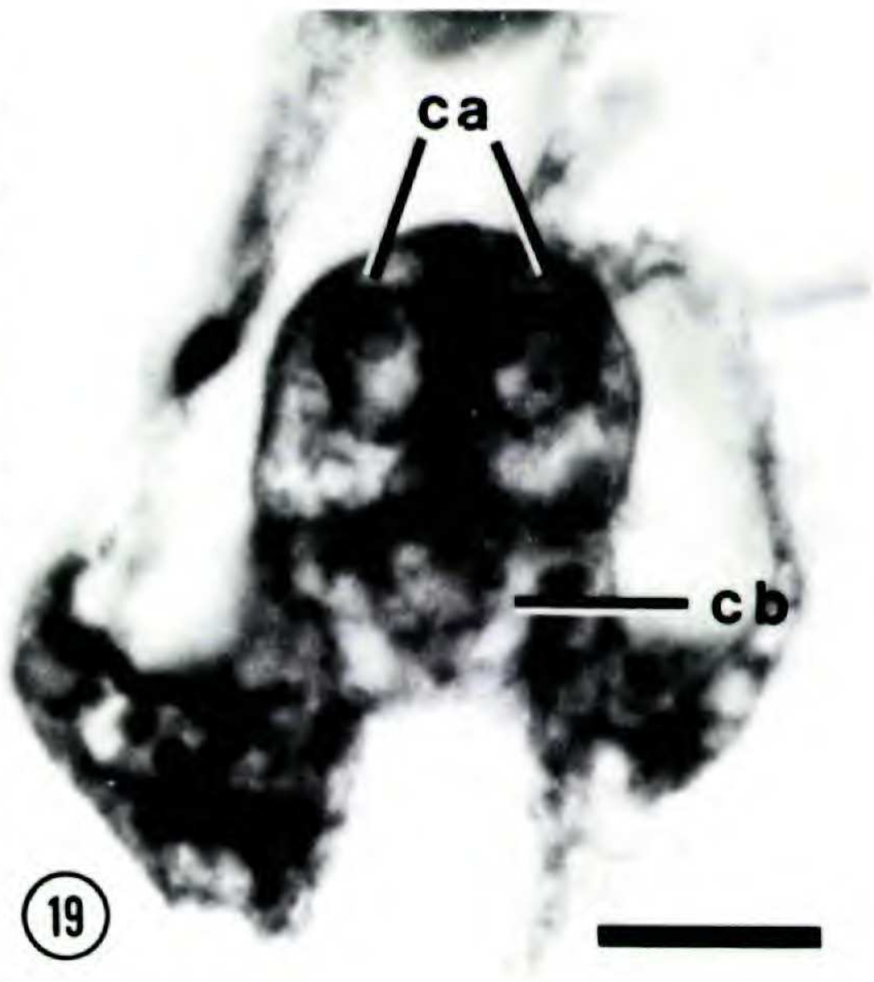
## FERTILIZATION, ENDOSPERM, AND EMBRYO (TABLE 5)

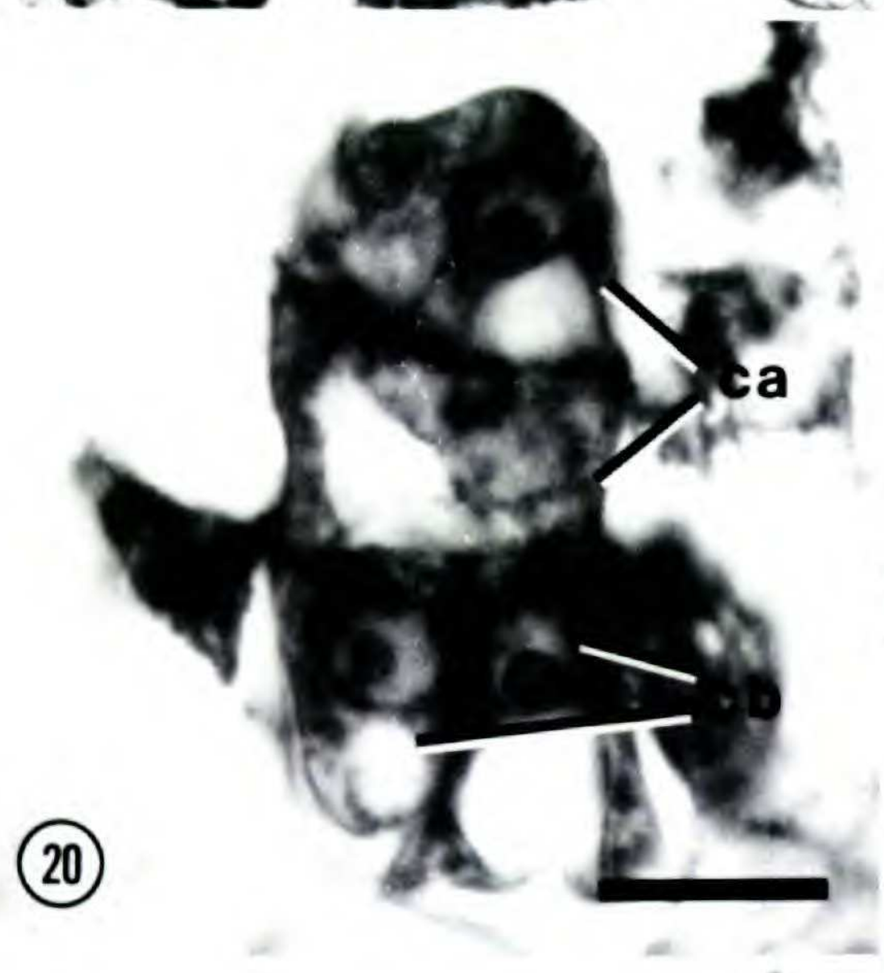
Fertilization in *Ludwigia* is porogamous. We often observed a remnant of a pollen tube penetrating into the micropyle (Fig. 17).

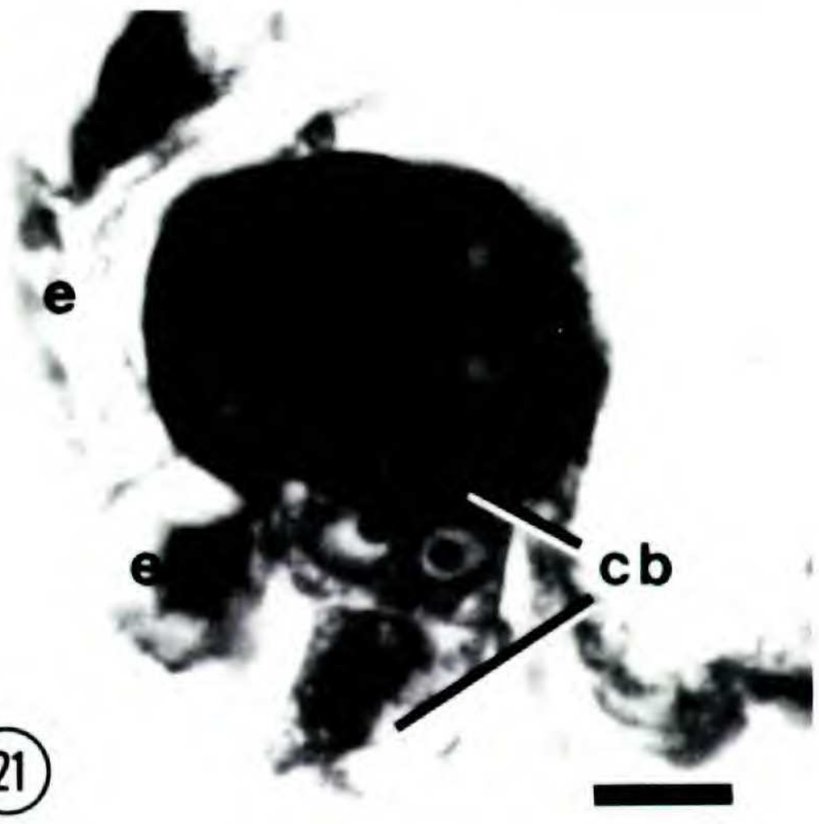
Endosperm formation is of the Nuclear type (Fig. 18). During the early, free-nuclear stage, the endosperm nuclei form separate groups on the micropylar and the chalazal sides of the embryo sac, rather than being scattered peripherally (Fig. 18). At this stage, the endosperm nuclei on the chalazal side characteristically have dense cytoplasm and form a very obvious group (Fig. 18). Wall formation in the free endosperm nuclei always begins in the micropylar group, ultimately reaching the chalazal one. Cellular endosperm probably never fills the embryo sac, because the endosperm is apparently absorbed by the growing embryo more quickly than it is formed. Mature seeds in all Onagraceae completely lack endosperm (Fig. 23).

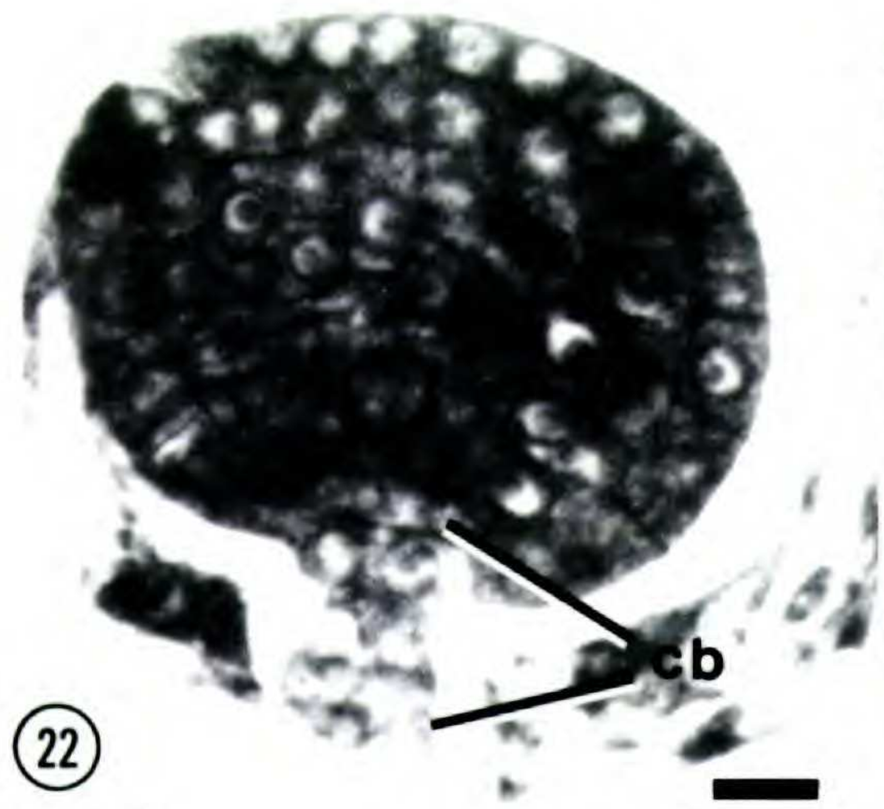
In a few species, we observed the details of embryogeny. *Ludwigia bonariensis, L. maritima,* and *L. glandulosa* all had the same Onagrad type embryogeny as reported in different species by all earlier workers (Souèges, 1935; Khan, 1942; Seshavataram, 1967, 1970). In *L. peploides* (sect. *Oligospermum*), however, the embryogeny was of the Solanad type. In both Onagrad and Solanad types, the basal cell of the two-celled proembryo plays only a minor or no part in the subsequent development of the embryo (Figs. 21, 22). The Solanad type differs from the Onagrad type in that the apical cell of the two-celled proembryo divides by a transverse wall (Fig. 20), instead of by a longitudinal wall as in the Onagrad type (Fig. 19). The suspensor is very short, mostly three or four cells long (Figs. 21, 22). The embryo in the mature seed is straight and has two equally developed cotyledons (Fig. 23).

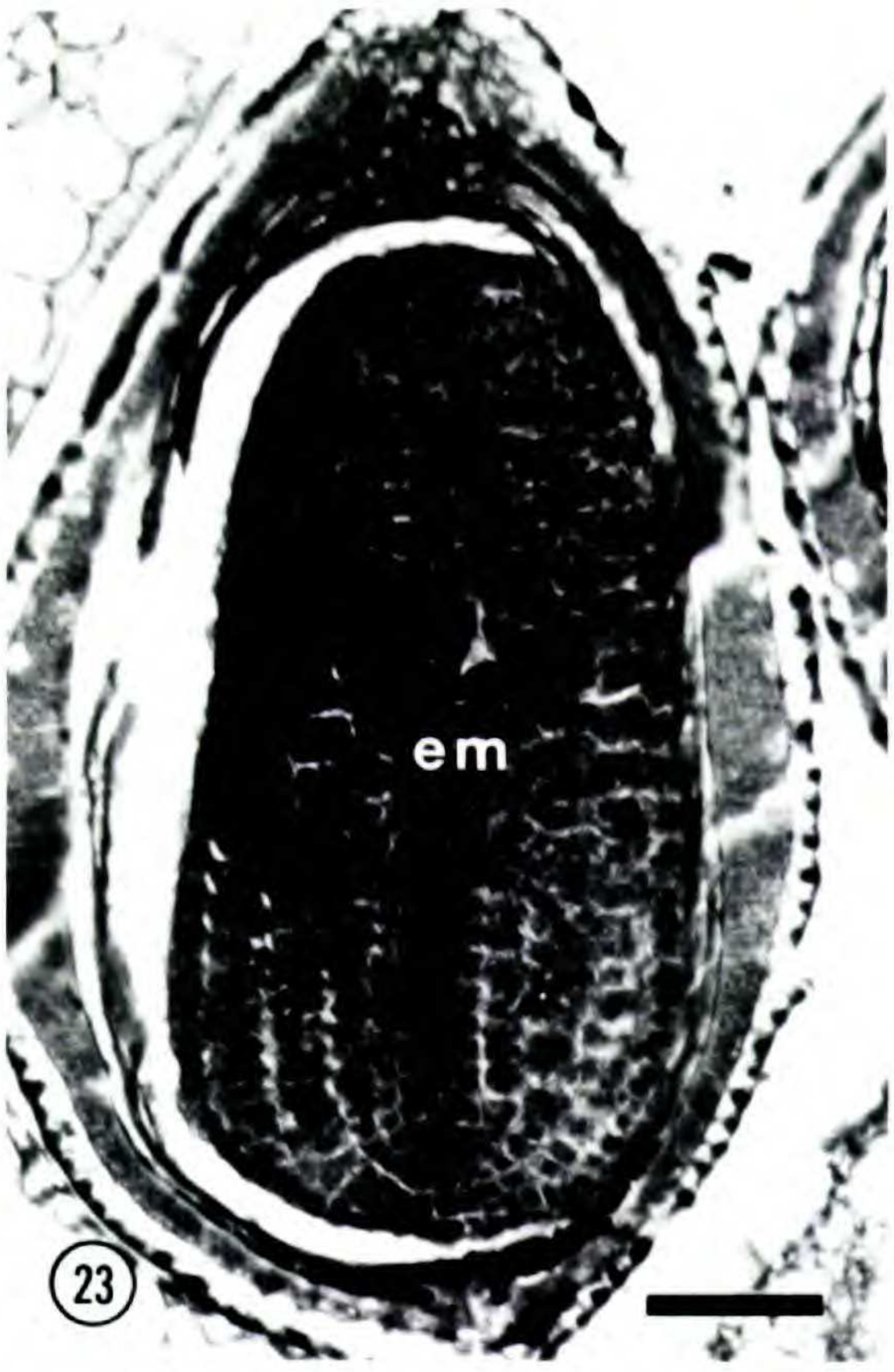



FIGURES 18–23. Endosperm formation and embryogeny in *Ludwigia*.—18. *L. maritima*. Longitudinal section (LS) of a young seed showing free endosperm nuclei (fe) and hypostase (hyp). Scale = 50 μm.—19. *L. maritima*.

### HYPOSTASE (TABLE 5)

In all of the species that we studied, we were able to confirm that the differentiation of the hypostase "consists of a well defined but irregularly outlined group of thick-walled cells at the chalazal end of the ovule" (Johansen, 1928a; see also Fig. 18). The hypostase was not observed, however, until the two- to four-celled proembryonal stage, at the earliest. In this respect, our results agree with those of Khan (1942) and Seshavataram (1968), who mentioned that the hypostase is not differentiated in younger ovules.

Starch grains appear in early megasporogenesis, whereas the hypostase cannot be distinguished until the early proembryonal stages. Consequently, the hypostase cannot, contrary to the views of Ishikawa (1918), play a role in the accumulation of starch grains.

### SEED COAT HISTOLOGY (TABLE 6)

Our comparisons are based on mature seeds that no longer contain endosperm, and on those portions of the seed coat that are formed of both inner and outer integuments; descriptions are based on longitudinal sections of mature seeds. In all of the species that we examined, the seed coat had basically the same histological structure, consisting of a two-layered tegmen and a two-layered testa (see Figs. 25–31; Corner, 1976; Eyde, 1978).

As regards the tegmen, all the species had similar cells in the exotegmen and the endotegmen, respectively. The cells of the exotegmen are characteristically elongate and tracheidal, with spiral wall thickenings. In contrast, the cells of the endotegmen are also elongate, but more or less collapsed. Ontogenetically, the cells of the endotegmen become elongate and tanniferous even prior to fertilization (Fig. 24). They may play a role in the early development of the seeds.

In contrast, there was considerable diversity in the structure of the testa, particularly in its thickness, within *Ludwigia*. The cells of the exotesta showed differences in cell substance as well as in size. The exotesta of *L. bonariensis* contained abundant tannins from the early stages of development onward (Figs. 24, 26). In most species, the cells of the exotesta were thin-walled, but those of *L. peploides* were exceptionally lignified and thick-walled (Fig. 31). The endotesta was found to be much more specialized than the exotesta. It was consistently lignified and composed of crystal cells in every species, and varied considerably in thickness from species to species, and from section to section. The endotestae of *L. peruviana* (sect. *Myrtocarpus*; Fig. 25), *L. bonariensis* (sect. *Macrocarpon*; Fig. 26), *L. maritima* (sect. *Ludwigia*; Fig. 27), *L. lanceolata* (sect. *Microcarpium*), *L. leptocarpa* (sect. *Seminuda*; Fig. 30), and *L. peploides* (sect. *Oligospermum*; Fig. 31) had approximately the same thickness throughout an individual seed. The endotestae of *L. repens* (Fig. 29) and *L. arcuata* (sect. *Dantia*) and those of *L. glandulosa* (sect. *Microcarpium*; Fig. 28) differed markedly in that they showed a difference in thickness from part to part: the middle part was thickest (i.e., about 38–44 μm thick), and the portions close to both ends were thinnest (i.e., about 8–17 μm thick).

## DISCUSSION

*Characteristics of* Ludwigia. This study has presented a considerable amount of new embryological data on *Ludwigia* and also critically reviewed the existing literature on the genus. In the light of this information, the embryological characteristics of *Ludwigia* may be summarized as follows:

Anther wall 5- to 6-layered; wall formation conforming to the Basic type; anther epidermis persistent; endothecium fibrous; two to three middle layers ephemeral; tapetum glandular, and its cell 2-nucleate. Cytokinesis in microspore mother cells simultaneous; microspores in a tet-

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Longitudinal section of a three-celled proembryo; apical cell of the two-celled proembryo and its derivatives (ca); basal cell of the two-celled proembryo (cb). Note that the apical cell was divided by a transverse wall. Scale = 10 μm.—20. *L. peploides*. Longitudinal section of a four-celled proembryo showing that the apical cell was divided by a transverse wall, and not by a longitudinal one. Symbols are the same as in 19. Scale = 10 μm.—21 and 22. *L. arcuata*. Longitudinal sections of successively older globular proembryos. Derivatives of the basal cell (cb) at the two-celled proembryo contribute only to a minor part of the embryo and mostly form a short suspensor. Endosperm (e) becomes cellular. Scales = 10 μm.—23. *L. repens*. LS of a mature seed showing a straight dictyledonous embryo (em), which occupies the whole space of the embryo sac. Note that endosperm is absent. Scale = 100 μm.

TABLE 6. A summary of embryological data on seed coat anatomy. Figures for thickness of endotesta in brackets indicate thickness of the thinnest portion of the layer.

| | Thickness of Coat Layers | | | | Type of Specialization | | | |
|---|---|---|---|---|---|---|---|---|
| Taxon | Tegmen (number of cell layers) | Testa (number of cell layers) | Endotesta ($\mu$m) | Exotesta ($\mu$m) | Endo-tegmen | Exotegmen | Endotesta | Exotesta |
| Sect. *Myrtocarpus* | | | | | | | | |
| *L. peruviana* | 2 | 2 | 10.6–12.7 | 8.5–10.6 | none | tracheidal | crystalliferous | none |
| Sect. *Macrocarpon* | | | | | | | | |
| *L. bonariensis* | 2 | 2 | 12.7–14.8 | 6.3–8.5 | none | tracheidal | crystalliferous | none |
| Sect. *Ludwigia* | | | | | | | | |
| *L. maritima* | 2 | 2 | 16.9–21.1 | 8.5–10.6 | none | tracheidal | crystalliferous | none |
| Sect. *Microcarpium* | | | | | | | | |
| *L. lanceolata* | 2 | 2 | 5.3–8.5 | 8.5–12.7 | none | tracheidal | crystalliferous | none |
| *L. glandulosa* subsp. *glandulosa* | 2 | 2 | 38.0–44.4 [14.8–16.9] | 14.8–21.1 | none | tracheidal | crystalliferous | none |
| Sect. *Dantia* | | | | | | | | |
| *L. repens* | 2 | 2 | 38.0–42.2 [8.5–12.7] | 12.7–16.9 | none | tracheidal | crystalliferous | none |
| Sect. *Seminuda* | | | | | | | | |
| *L. leptocarpa* | 2 | 2 | 23.2–31.7 | 19.0–21.1 | none | tracheidal | crystalliferous | none |
| Sect. *Oligospermum* | | | | | | | | |
| *L. peploides* | 2 | 2 (3) | 7.4–10.6 | 4.2–6.3 | none | tracheidal | crystalliferous | none |

rahedral, decussate or isobilateral tetrad; pollen grains 2-celled when shed.

Ovule anatropous, crassinucellate and bitegmic; inner integument 2-layered and outer integument also 2-layered, rarely 3-layered (*Ludwigia peploides*); micropyle formed by both integuments.

Archesporium one-celled; archesporial cell cutting off a primary parietal cell and forming a parietal tissue three to six cell layers thick; meiosis in megaspore mother cells resulting usually in a linear tetrad of megaspores, occasionally in a linear triad; micropylar megaspore functional, developing into a 4-nucleate *Oenothera* type embryo sac, comprising an egg, two synergids, and one polar nucleus; apical nucellar epidermal cells not dividing periclinally; prominent accumulation of starch grains in the nucellus common.

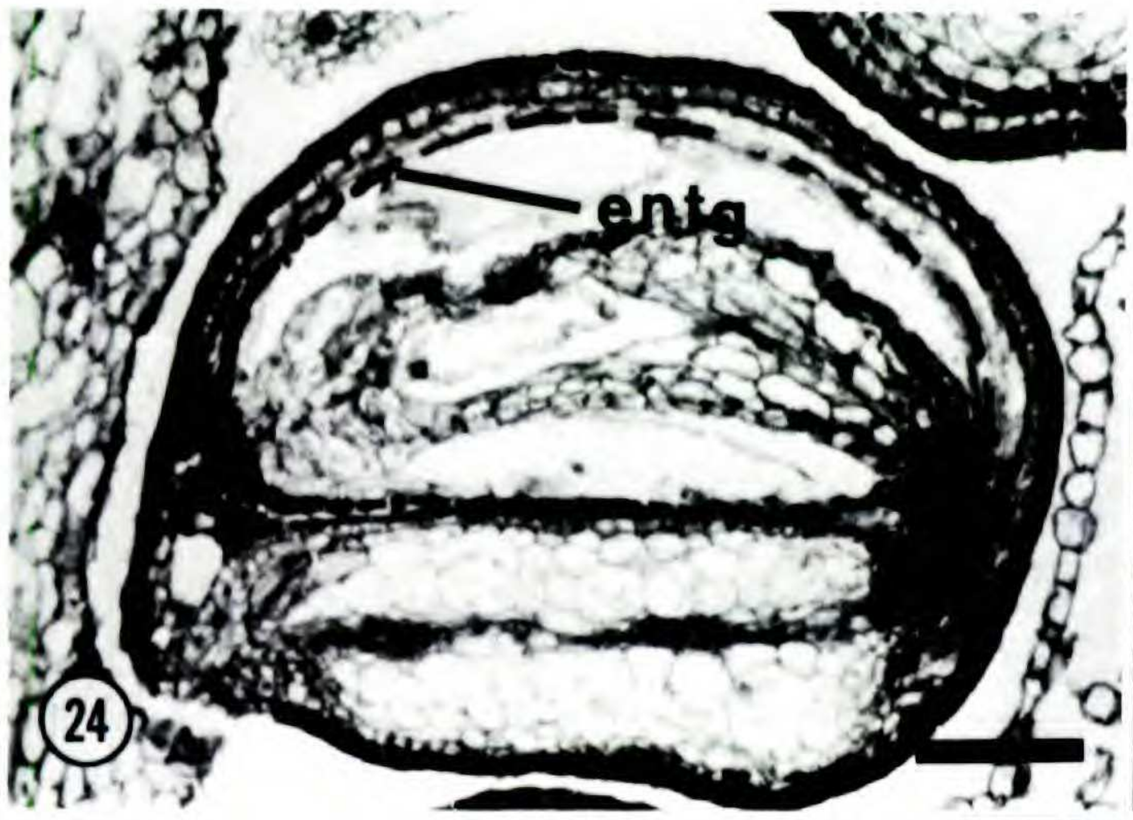
Fertilization porogamous; endosperm formation of the Nuclear type; free-nuclear endosperm becoming cellular later; seed exalbuminous; embryogeny conforming mostly to the Onagrad type, rarely to the Solanad type (*Ludwigia peploides*); suspensor short; embryo straight and with two equally developed cotyledons; hypostase differentiated in proembryonal stages.

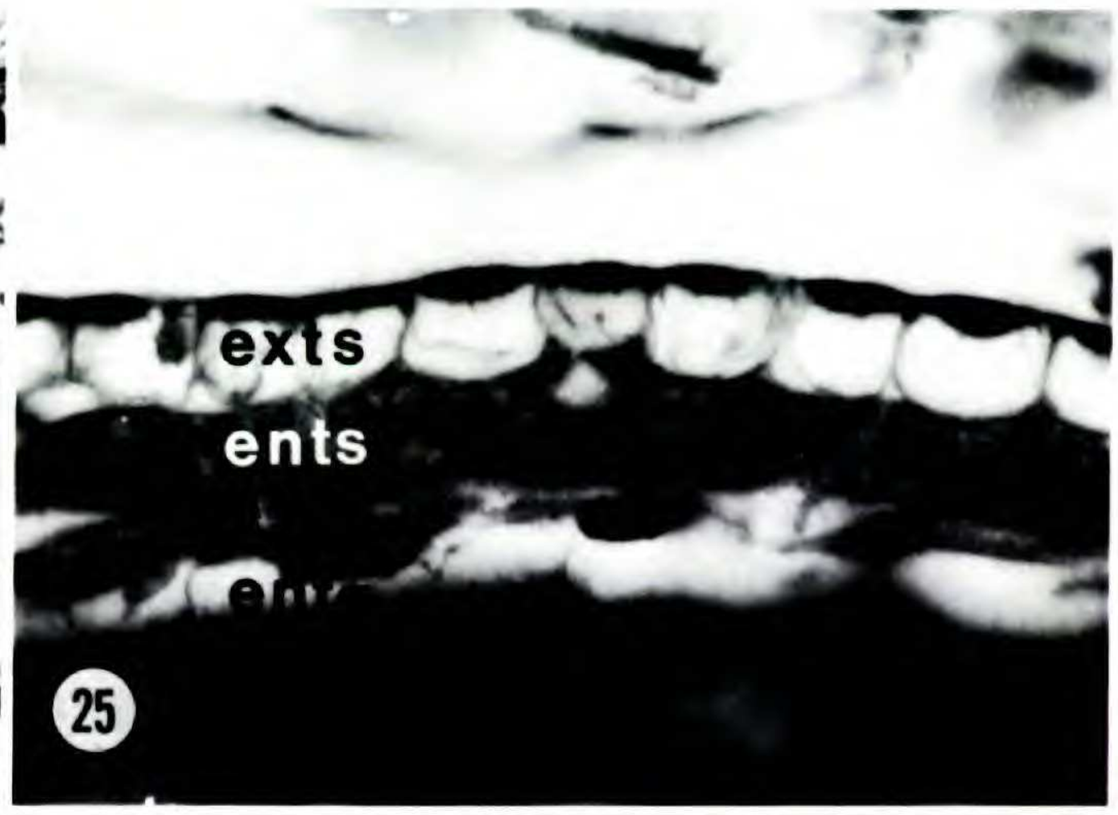
Seed coat composed of 2-layered tegmen and 2-layered testa; exotegmen tracheidal; endotesta crystalliferous, varying in thickness.

*Relationships of sections* Microcarpium *and* Dantia. In this discussion, we shall consider only those characteristics that vary within the genus as an index of relationships. As seen in Tables 2–6, however, there are relatively few such characteristics among the embryological features of *Ludwigia*. Furthermore, some of the characteristics that do vary within the genus (e.g., thickness of the anther wall, thickness of the nucellar parietal tissue, thickness of the seed coat, shape of mature embryo sac, and type of embryogeny) do not fall into distinct classes clearly enough to distinguish species or sections from one another. The most diversified and presumably significant character is the histology of the seed coat, which differs from section to section in the degree of specialization of its constituent layers—particularly that of the endotesta.

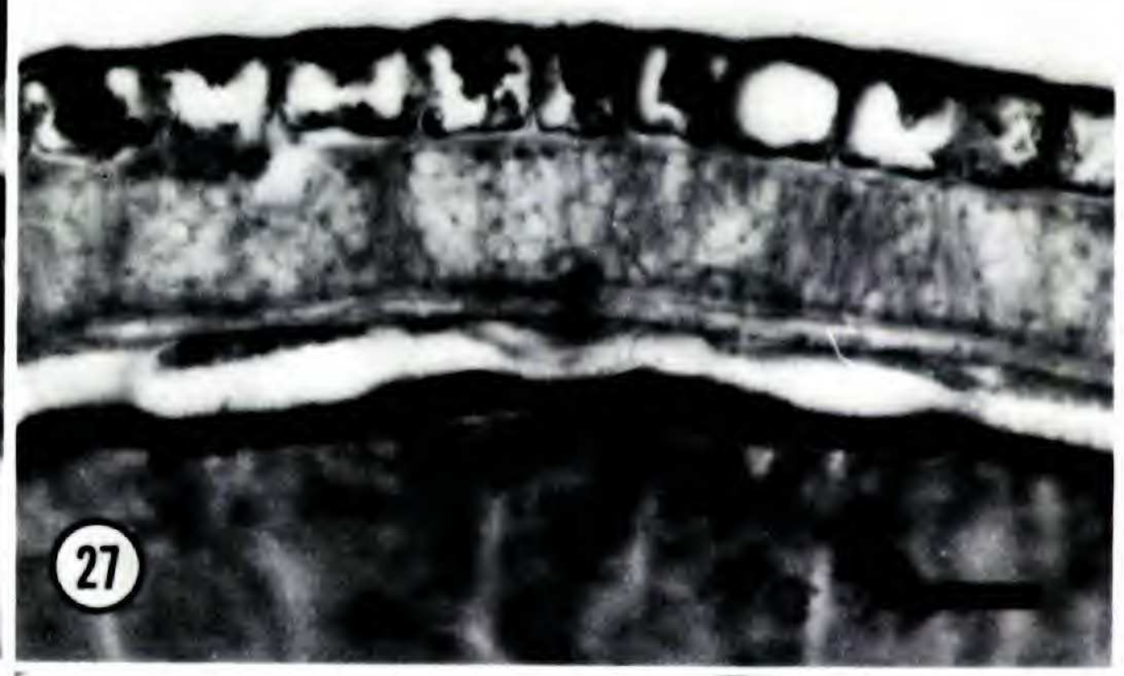
The seed coats of many sections (i.e., *Ludwigia, Macrocarpon, Myrtocarpus, Oligospermum,* and *Seminuda*) exhibit a condition that we regard as generalized; in it, all of the constituent layers are of uniform thickness over the entire seed. We consider structure of this kind to be basic because of its wide distribution in *Ludwigia*. In contrast, sect. *Dantia* exhibits a more specialized structure, in which the endotesta is much thicker at the median part of the seed than at the two ends; the thickest part of the endotesta in species of sect. *Dantia* is as much as 38–44 μm thick (see Table 6). We examined two of the five species of sect. *Dantia,* and Eyde (1978) obtained the same results for a third. In sect. *Microcarpium,* one of the two species examined, *L. lanceolata,* had a seed coat of uniform thickness, whereas another, *L. glandulosa,* had a structure similar to that of sect. *Dantia*. We have checked this character in the other species of sect. *Microcarpium,* and found that some of them (i.e., *L. alata, L. linearis, L. pilosa, L. polycarpa, L. ravenii,* and *L. sphaerocarpa*) have a more or less thin uniform endotesta about 4–16 μm thick, whereas the others (i.e., *L. curtisii, L. linifolia, L. microcarpa, L. simpsonii,* and *L. stricta*) have a thicker endotesta similar to that of sect. *Dantia*.

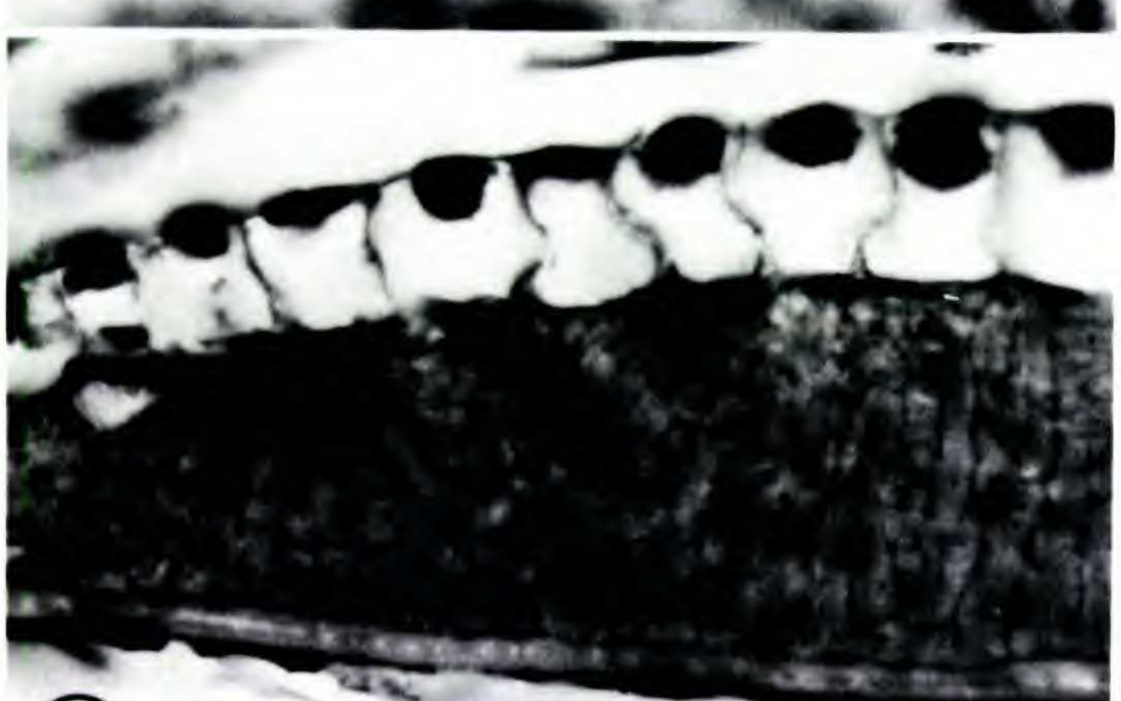
Earlier students, basing their conclusions on other features, pointed out that sect. *Microcarpium* is diverse (see Raven & Tai, 1979). Species differ in seed surface pattern (Eyde, 1978; Peng, 1982), floral vasculature (Eyde, 1981), mode of capsule dehiscence (Peng & Tobe, 1987), histological structure of the capsule wall (Peng & Tobe, 1987), and whether the pollen is shed singly or in tetrads (Raven, 1963). Overall, sects. *Dantia* and *Microcarpium* appear to be closely related (see Raven, 1963; Eyde, 1978, 1981), and hybrids between species of these two sections are easily obtained and fairly frequent in nature (Schmidt, 1967; Peng, 1982). The evidently derived seed coat structure found in six of the 13 species of sect. *Microcarpium* and at least three of the five species of sect. *Dantia* also suggests that these groups are closely related; the kind of specialized seed coat found in these groups is unknown elsewhere in the genus. Eyde (1978: 663) implied that the seed coat structure of these two sections differed, stating that "seeds of sections *Dantia* and *Microcarpium* are histologically similar to those of section *Myrtocarpus,* but in *Ludwigia palustris* (i.e., sect. *Dantia*) the cells of the crystalliferous layer can differ greatly in size from one part of the seed to another, those in the middle of the seed being much larger than those of the ends." We have found, however, that six of the 13 species of sect. *Microcarpium* are identical to sect. *Dantia* in seed coat structure. Different species of sect. *Microcarpium,* although they are clearly related to one another, have both



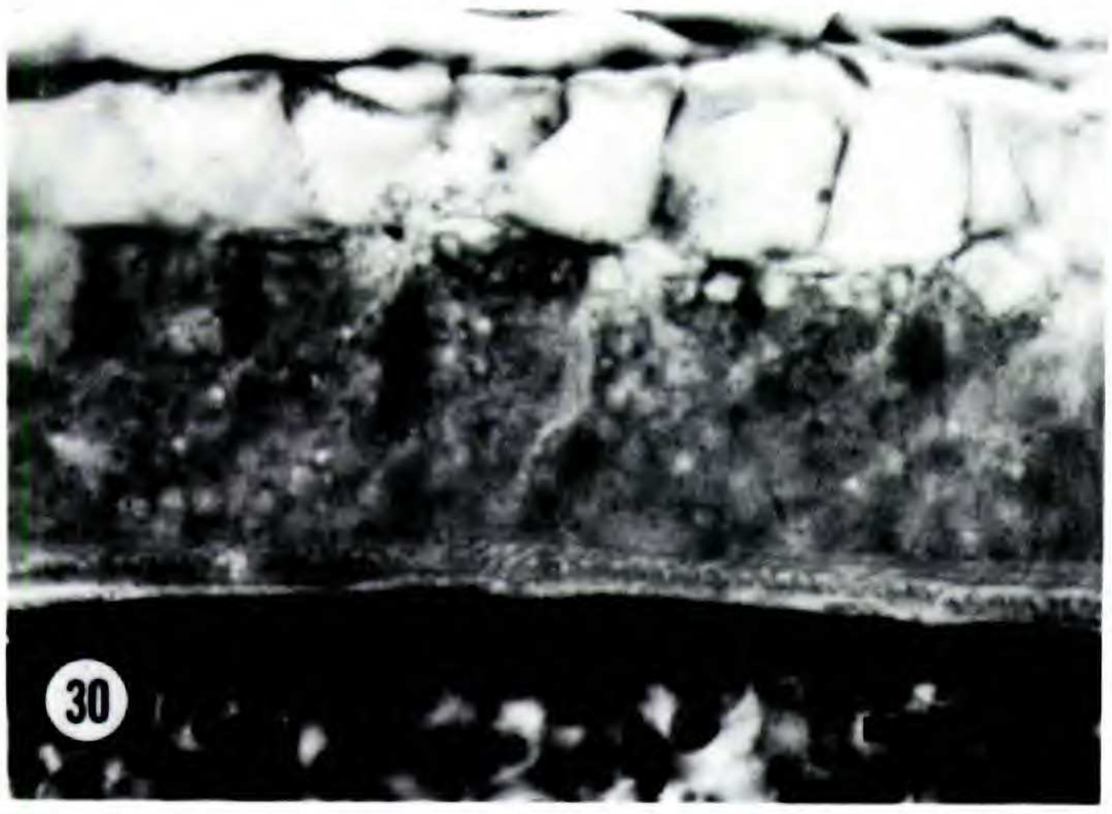



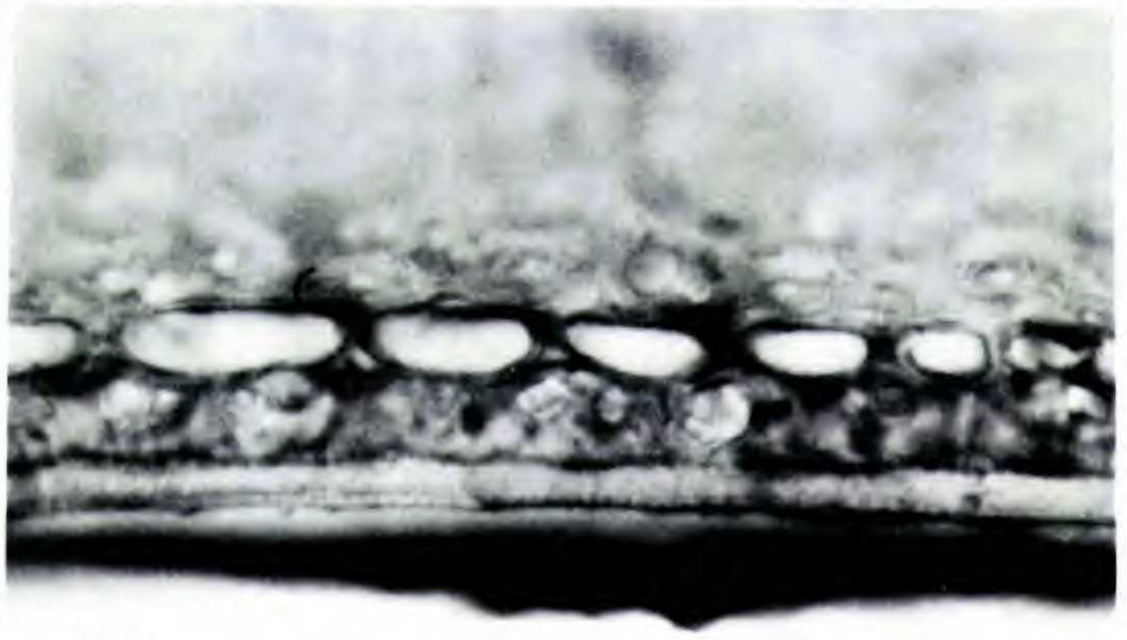









FIGURES 24–31. Seed coat structure of *Ludwigia*.—24. *L. bonariensis*. Longitudinal section of a young seed showing a tanniferous elongate endotegmen (entg). Scale = 100 μm.—25–31. Longitudinal sections of mature

the generalized and the derived types of seed coat structure, a relationship that makes it almost certain that the specialization of the seed coat structure occurred within this group. Thus by the occurrence in sect. *Dantia* of two synapomorphies—the specialized seed coat and its entirely opposite leaves—that section is clearly derived in the context of *Ludwigia* as a whole.

*Relationships with other Onagraceae and Myrtales.* In contrast with the traditional view, which holds that *Ludwigia* is the primitive genus closest to the prototype of Onagraceae and therefore the best link with Lythraceae and other Myrtales (see Melchior, 1964; Takhtajan, 1980), recent investigations have made it increasingly clear that *Ludwigia* is, in fact, the sister group of all other Onagraceae (Eyde, 1981: 404; see also Raven & Tai, 1979; Eyde, 1977, 1978). It therefore represents a distinct evolutionary line, but would by no means be expected to have a monopoly on primitive features within the family.

Nonetheless, a comparison of *Ludwigia* with other Onagraceae affords us one important way to evaluate the characteristics of their common ancestor. For example, that *Ludwigia* has the typical distinctive 4-nucleate *Oenothera* type embryo sac like all other Onagraceae clearly indicates that this feature evolved in the ancestor of the family or its derivative before the evolutionary line leading to *Ludwigia* diverged from that leading to the rest of the family.

Earlier embryologists (Tischler, 1917; Mauritzon, 1934; Joshi & Venkateswarlu, 1937) repeatedly suggested that the *Oenothera* type embryo sac might have been derived from the normal-type embryo sac of Lythraceae, which like *Oenothera* lacked antipodal cells at maturity because they were ephemeral. We now know, however, that ephemeral antipodal cells are characteristic of most Myrtales and do not, therefore, support a hypothesis of a direct relationship between Onagraceae and Lythraceae (Tobe & Raven, 1983); moreover, the lack of antipodal cells in mature embryo sacs has a completely different basis in Onagraceae, where they are never formed, from that in other Myrtales, where they are formed and then lost. The comparison is a false one, based on superficial convergence.

The *Oenothera* type embryo sac is quite different from the normal-type embryo sacs in the following two respects: (1) the micropylar megaspore in a tetrad, instead of the chalazal one in the case of the normal type, always functions to develop into an embryo sac; (2) the nucleus of the functional megaspore always divides twice, instead of three times, which results in a 4-nucleate embryo sac. With respect to a reversed polarity of the functional megaspore, Rodkiewicz and Bednara (1974) have postulated that the uneven distribution of starch grains and dictyosomes in megaspores as well as of callose on megaspore walls may prevent the development of the chalazal megaspore into an embryo sac. Starch grains accumulate in the micropylar megaspore but not to as great an extent as in the chalazal one; dictyosomes occur at a greater density in the chalazal megaspore than in the micropylar one; and callose is laid down on all megaspore walls except for the upper wall of the micropylar megaspore.

The kind of embryo sac that is characteristic of Onagraceae, which has only four nuclei, might be regarded as a result of neoteny: functioning at an earlier stage of development than is characteristic of most plants. The several substantial differences between the *Oenothera* type of embryo sac and that characteristic of most angiosperms suggest that the former may have originated as a result of the accumulation of several different mutations. It is probably because of the complex nature of these differences that the *Oenothera* type embryo sac is unknown except in Onagraceae. Judged from its ubiquity in the family, this unique type of embryo sac must have been present in the common ancestor of the two fundamentally different lines leading, respectively, to *Ludwigia* and to the rest of the family.

Onagraceae resemble Lythraceae in a number of features, including wood anatomy (Carlquist, 1975), leaf architecture (Hickey, pers. comm., in Dahlgren & Thorne, 1984), seed coat histology (Corner, 1976), and petal venation pattern (Chrtek, 1969). Our results confirm that these two families share a specialized tracheidal exotegmen, which, however, is also found in two unrelated families of Myrtales; i.e., Combreta-

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seed coats, each of which is composed of a two-layered testa and a two-layered tegmen; exotesta (exts); endotesta (ents); exotegmen (extg); endotegmen (entg). Scales = 20 μm.—25. *L. peruviana.*—26. *L. bonariensis.*—27. *L. maritima.*—28. *L. glandulosa.*—29. *L. repens.*—30. *L. leptocarpa.*—31. *L. peploides.*

ceae and Trapaceae (Corner, 1976). In Myrtales generally, starch grains accumulate in the nucellus only in Onagraceae and Lythraceae. Ishikawa (1918) reported abundant starch grains in *Oenothera, Gaura,* and *Circaea* (Onagraceae), and Hubert (1896) in *Cuphea* (Lythraceae). We here report their ubiquitous occurrence in *Ludwigia* as well.

We are at present studying the embryology of all other 16 genera of Onagraceae as well as of some Lythraceae. Further detailed comparisons will be presented in subsequent papers.

*Embryology and relationships within* Ludwigia. Many past studies of embryology have been too limited in scope to warrant the general conclusions that have been drawn from them. Most have been concerned only with the development of the male and female gametophyte and that of the embryo, and the number of actual observations made have often been too few to warrant the generalizations that have been based on them. Our present study has been based on a sufficient number of species to represent the range of variation of the genus and has included, insofar as possible, all of its embryological features. In the light of this information, it is possible to offer some suggestions about relationships within *Ludwigia,* based on our observations.

Most embryological features in *Ludwigia* did not vary throughout the genus. However, differences in two important features have been noted within the genus, and these are the type of embryogeny and the structure of the endotesta. Along with earlier students of *Ludwigia* embryology, we found that most species we examined had the Onagrad type embryogeny. The only exception was *L. peploides* (sect. *Oligospermum*), which had the Solanad type embryogeny. This finding agrees with other evidence in suggesting an isolated position for sect. *Oligospermum* within the genus, although the earlier report of Onagrad type embryogeny in *L. adscendens* (= "*Jussieua repens*"; Khan, 1942), a species that is very closely related to *L. peploides,* needs to be investigated. Embryogeny often varies considerably at a family level (see Davis, 1966: 25–26), and a sufficient number of species need to be investigated before conclusions are drawn about this feature.

As we mentioned above, the species of *Ludwigia* sect. *Dantia* and some species of sect. *Microcarpium* have a more specialized endotestal structure than the rest of sect. *Microcarpium* and the other species of the genus. Based on the occurrence of this clearly apomorphic feature, it seems certain not only that the groups are directly related, but that sect. *Dantia,* with its opposite leaves—another clearly apomorphic feature, unique for *Ludwigia*—was derived from an ancestor that would, if it were known, be placed in sect. *Microcarpium.* Although diverse, sect. *Microcarpium* seems clearly to consist of elements that are directly related to one another.

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